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Doudna et al.

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(45) **Date of Patent: Sep. 6, 2016**

(54) **METHOD OF PRODUCING DICER**

(71) Applicant: **The Regents of the University of California**, Oakland, CA (US)

(72) Inventors: **Jennifer A. Doudna**, Berkeley, CA (US); **Enbo Ma**, Moraga, CA (US)

(73) Assignee: **THE REGENTS OF THE UNIVERSITY OF CALIFORNIA**, Oakland, CA (US)

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(21) Appl. No.: **14/468,109**

(22) Filed: **Aug. 25, 2014**

(65) **Prior Publication Data**

US 2015/0361406 A1 Dec. 17, 2015

Related U.S. Application Data

(62) Division of application No. 13/565,453, filed on Aug. 2, 2012, now Pat. No. 8,852,911.

(60) Provisional application No. 61/515,647, filed on Aug. 5, 2011, provisional application No. 61/515,135, filed on Aug. 4, 2011.

(51) **Int. Cl.**

C12N 9/22	(2006.01)
C12N 9/16	(2006.01)
C12N 15/11	(2006.01)
C12N 15/113	(2010.01)

(52) **U.S. Cl.**

CPC . ***C12N 9/22*** (2013.01); ***C12N 9/16*** (2013.01); ***C12N 15/111*** (2013.01); ***C12N 15/113*** (2013.01); ***C12Y 301/26003*** (2013.01); *C12N 2310/14* (2013.01); *C12N 2330/00* (2013.01); *C12N 2330/50* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Paul Holland

(74) *Attorney, Agent, or Firm* — Bozicevic, Field & Francis, LLP; Paula A. Borden; Kyle A. Gurley

(57) **ABSTRACT**

The present disclosure provides a method for producing a Dicer polypeptide in a prokaryotic host cell. The present disclosure further provides a purified Dicer complex. The present disclosure further provides kits for producing a Dicer polypeptide in a prokaryotic host cell.

9 Claims, 22 Drawing Sheets

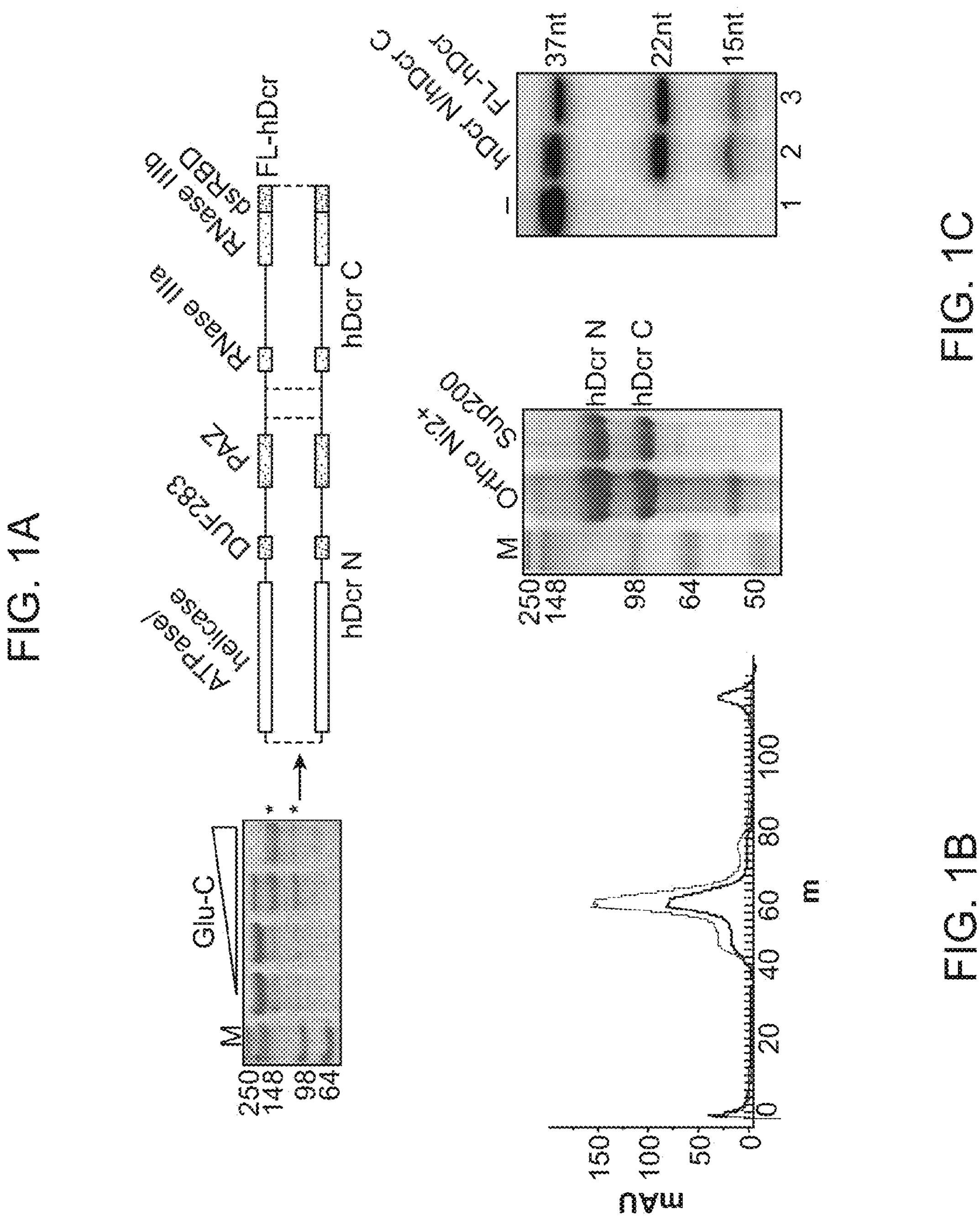


FIG. 1A

FIG. 1B

FIG. 1C

FIG. 2A

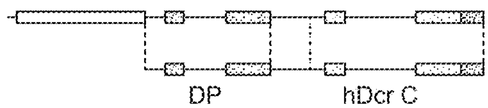


FIG. 2B

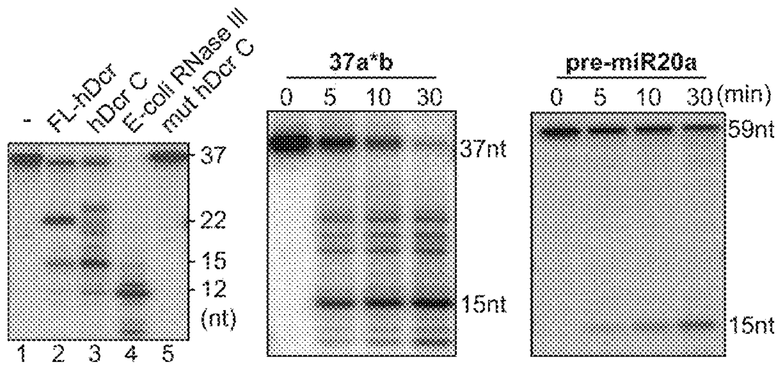


FIG. 2C

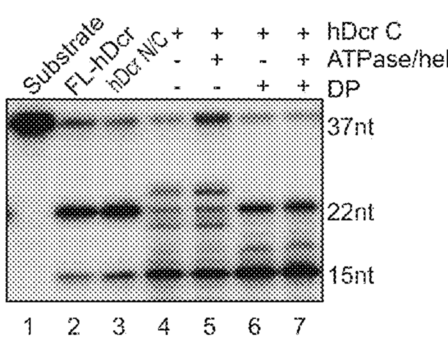


FIG. 3A

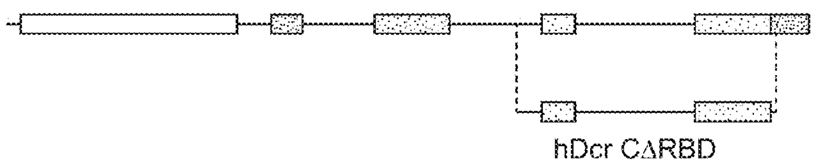


FIG. 3B

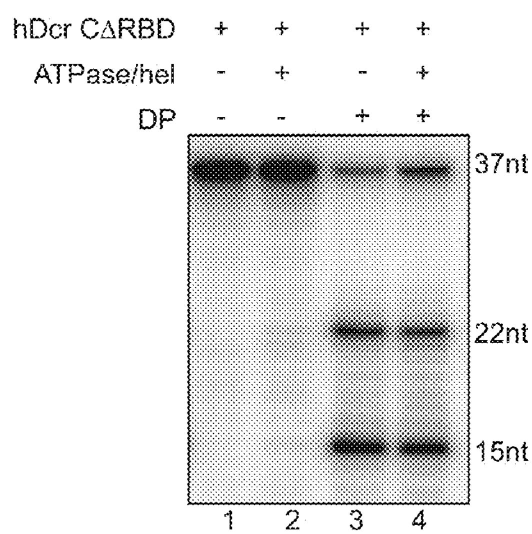


FIG. 4A

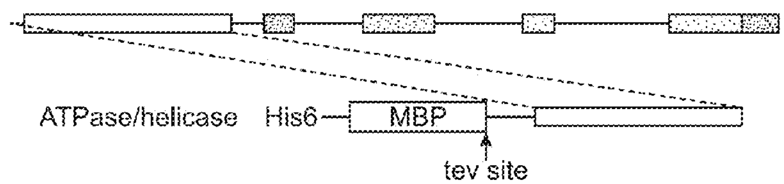


FIG. 4B

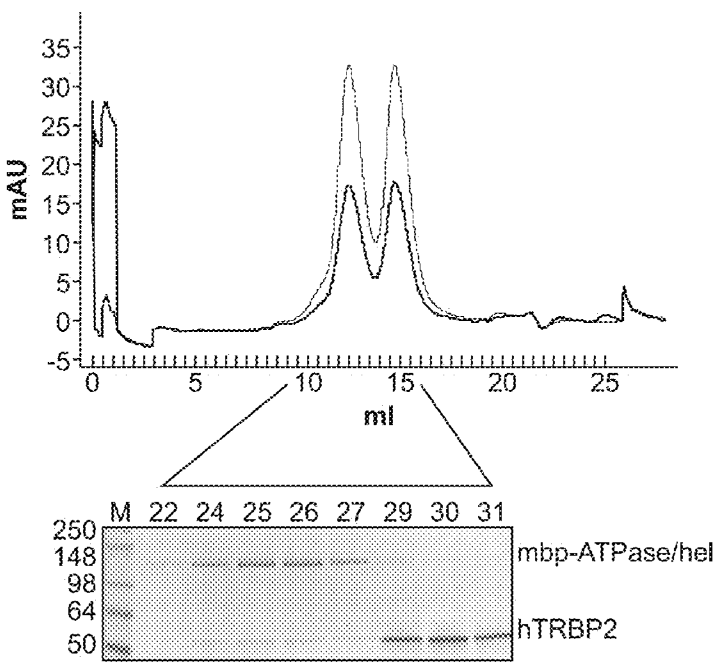


FIG. 4C

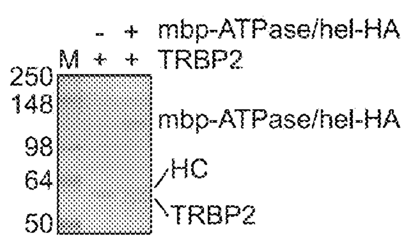


FIG. 5A

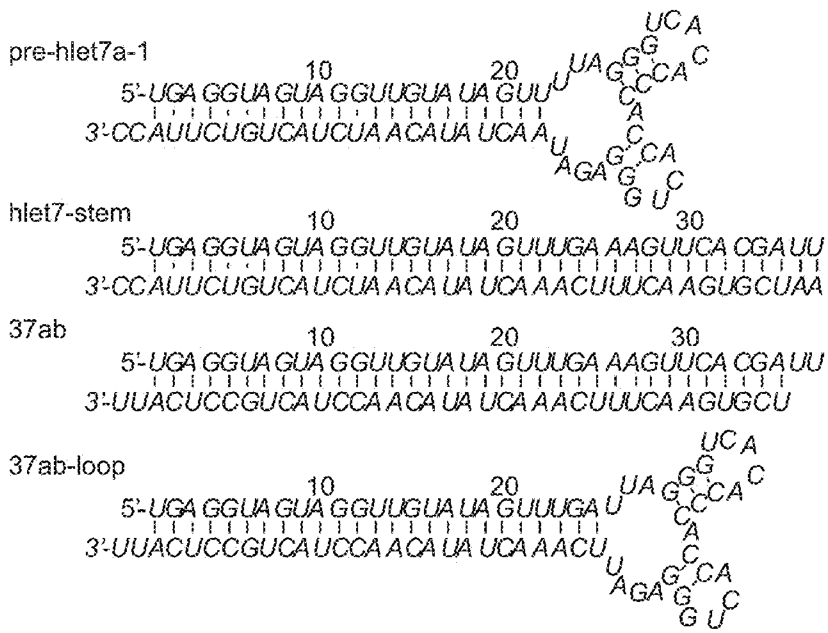


FIG. 5B

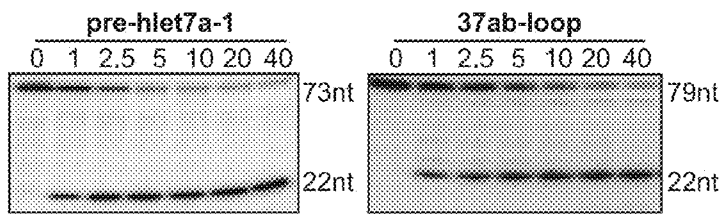
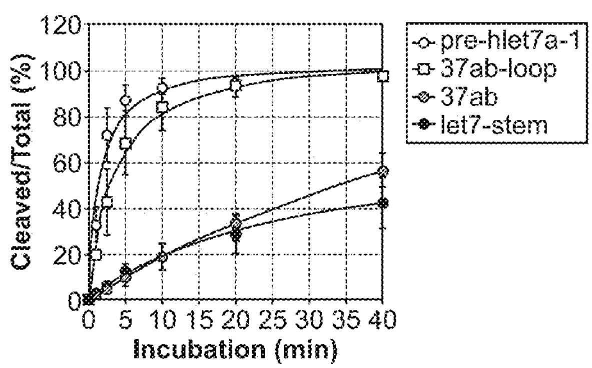


FIG. 5C



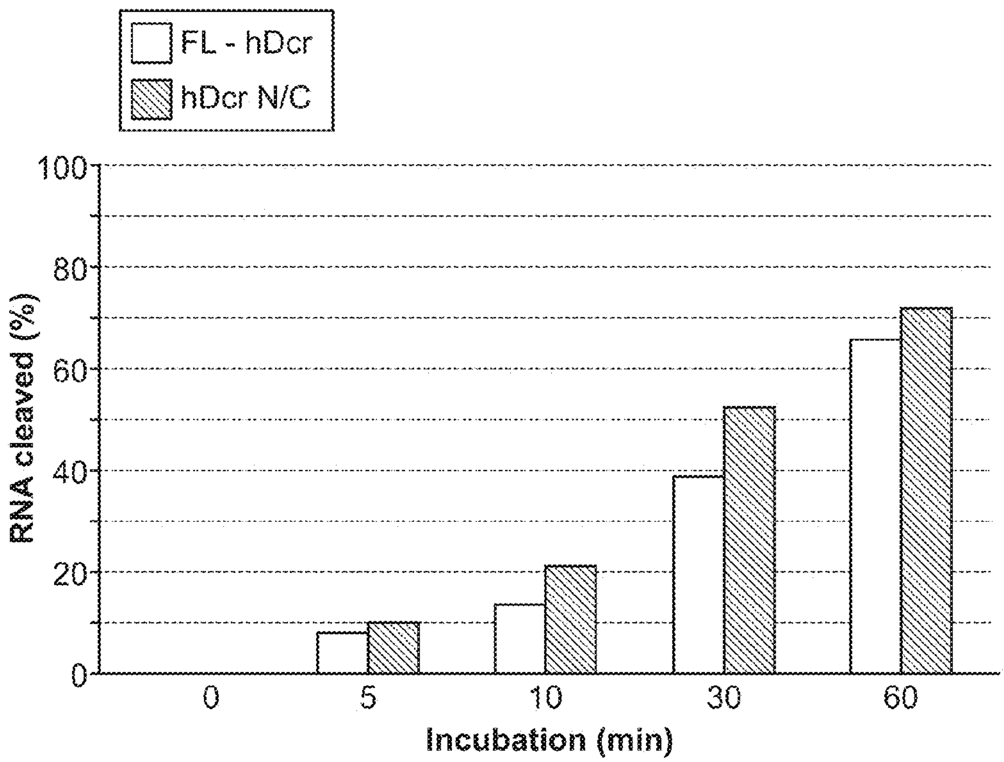

FL - hDcr
hDcr N/C
RNA cleaved (%)
100
80
60
40
20
0
0
5
10
30
60
Incubation (min)

FIG. 6

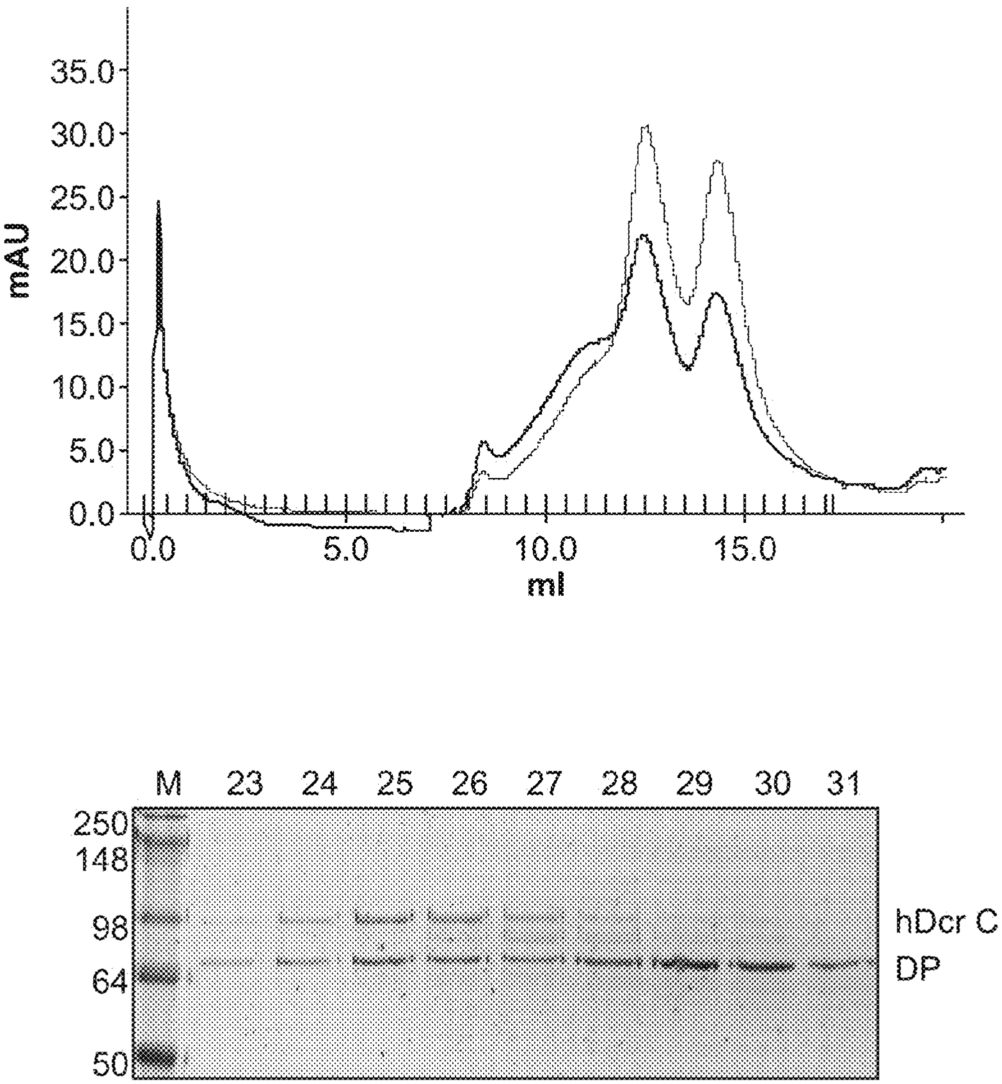

35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
mAU
0.0
5.0
10.0
15.0
ml
M
23
24
25
26
27
28
29
30
31
250
148
98
64
50
hDcr C
DP

FIG. 7

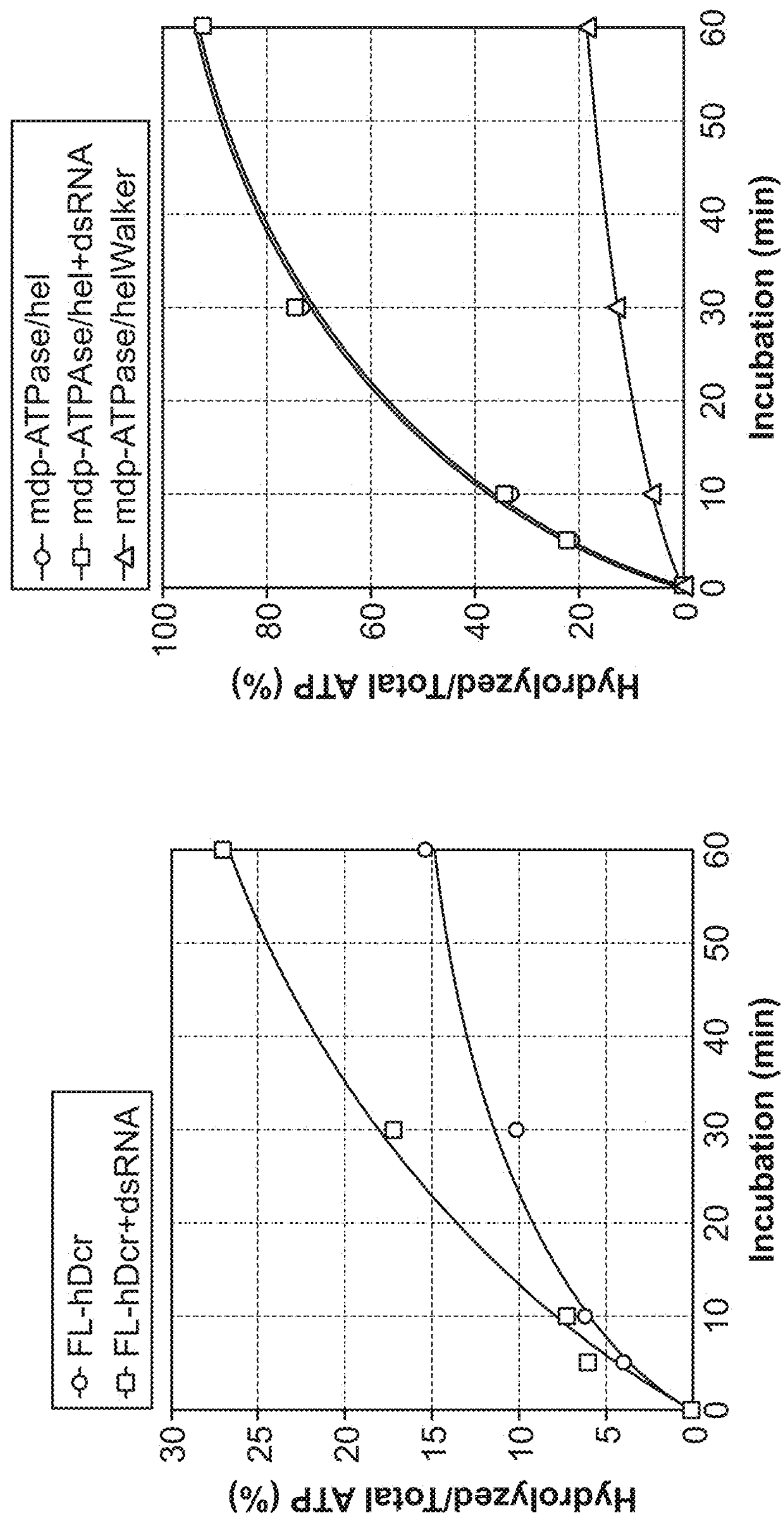

mdp-ATPase/hel
mdp-ATPase/hel+dsRNA
mdp-ATPase/helWalker
Hydrolyzed/Total ATP (%)
Incubation (min)
FL-hDcr
FL-hDcr+dsRNA
Hydrolyzed/Total ATP (%)
Incubation (min)

FIG. 8

Human Dicer 1

GenBank NP_803187

Homo sapiens

```
   1 mkspalqpls maglqlmtpa sspmgpffgl pwqqeaihdn iytprkyqve lleaaldhnt
  61 ivclntgsgk tfiavlltke lsyqirgdfs rngkrtvflv nsanqvaqqv savrthsdlk
 121 vgeysnlevn aswtkerwnq eftkhqvlim tcyvalnvlk ngylslsdin llvfdechla
 181 ildhpyreim klcencpscp rilgltasil ngkcdpeele ekiqklekil ksnaetatdl
 241 vvldrytsqp ceivvdcgpf tdrsglyerl lmeleealnf indcnisvhs kerdstlisk
 301 qilsdcravl vvlgpwcadk vagmmvrelq kyikheqeel hrkfllftdt flrkihalce
 361 ehfspasldl kfvtpkvikl leilrkykpy erqqfesvew ynnrnqdnyv swsdseddde
 421 deeieekekp etnfpspftn ilcgiifver rytavvlnrl ikeagkqdpe layissnfit
 481 ghgigknqpr nkqmeaefrk qeevlrkfra hetnlliats iveegvdipk cnlvvrfdlp
 541 teyrsyvqsk grarapisny imladtdkik sfeedlktyk aiekilrnkc sksvdtgetd
 601 idpvmddddv fppyvlrpdd ggprvtinta ighinrycar lpsdpfthla pkcrtrelpd
 661 gtfystlylp insplrasiv gppmscvrla ervvalicce klhkigeldd hlmpvgketv
 721 kyeeeldlhd eeetsvpgrp gstkrrqcyp kaipeclrds yprpdqpcyl yvigmvlttp
 781 lpdelnfrrr klyppedttr cfgiltakpi pqiphfpvyt rsgevtisie lkksgfmlsl
 841 qmlelitrlh qyifshilrl ekpalefkpt dadsaycvlp lnvvndsstl didfkfmedi
 901 eksearigip stkytketpf vfkledyqda viipryrnfd qphrfyvadv ytdltplskf
 961 pspeyetfae yyktkynldl tnlnqplldv dhtssrlnll tprhlnqkgk alplssaekr
1021 kakweslqnk qilvpelcai hpipaslwrk avclpsilyr lhclltaeel raqtasdagv
1081 gvrslpadfr ypnldfgwkk sidsksfisi snsssaendn yckhstivpe naahqganrt
```

FIG. 9

```
1141 sslenhdqms vncrtllses pgklhvevsa dltainglsy nqnlangsyd lanrdfcqgn
1201 qlnyykqeip vqpttsysiq nlysyenqpq psdectllsn kyldgnanks tsdgspvmav
1261 mpgttdtiqv lkgrmdseqs psigyssrtl gpnpglilqa ltlsnasdgf nlerlemlgd
1321 sflkhaitty lfctypdahe grlsymrskk vsncnlyrlg kkkglpsrmv vsifdppvnw
1381 lppgyvvnqd ksntdkwekd emtkdcmlan gkldedyeee deeeeslmwr apkeeadyed
1441 dfleydqehi rfidnmlmgs gafvkkisls pfsttdsaye wkmpkksslg smpfssdfed
1501 fdysswdamc yldpskavee ddfvvgfwnp seencgvdtg kqsisydlht eqciadksia
1561 dcveallgcy ltscgeraaq lflcslglkv lpvikrtdre kalcptrenf nsqqknlsvs
1621 caaasvassr ssvlkdseyg clkipprcmf dhpdadktln hlisgfenfe kkinyrfknk
1681 ayllqaftha syhyntitdc yqrleflgda ildylitkhl yedprqhspg vltdlrsalv
1741 nntifaslav kydyhkyfka vspelfhvid dfvqfqlekn emqgmdselr rseedeekee
1801 dievpkamgd ifeslagaiy mdsgmsletv wqvyypmmrp liekfsanvp rspvrellem
1861 epetakfspa ertydgkvrv tvevvgkgkf kgvgrsyria ksaaarralr slkanqpqvp
1921 ns
```

(SEQ ID NO:1)

FIG. 9 (Cont.)

DexD/H-box

```
  1 mkspalqpls maglqlmtpa sspmgpffgl pwqqeaihdn iytprkyqve lleaaldhnt
 61 ivclntgsgk tfiavlltke lsyqirgdfs rngkrtvflv nsanqvaqqv savrthsdlk
121 vgeysnlevn aswtkerwnq eftkhqvlim tcyvalnvlk ngylslsdin llvfdechla
181 ildhpyreim klcencpscp rilgltasil ngkcdpeele ekiqklekil ksnaetatdl
241 vvldrytsqp ceivvdcgpf tdrsglyerl lmeleealnf indcnisvhs kerdstlisk
301 qilsdcravl vvlgpwcadk vagmmvrelq kyikheqeel hrkfllftdt flrkihalce
361 ehfspasldl kfvtpkvikl leilrkykpy erqqfesvew ynnrnqdnyv swsdseddde
421 deeieekekp etnfpspftn ilcgiifver rytavvlnrl ikeagkqdpe layissnfit
481 ghgigknqpr nkqmeaefrk qeevlrkfra hetnlliats iveegvdipk cnlvvrfdlp
541 teyrsyvqsk grarapisny imladtdkik sfeedlktyk aiekilrnkc sksvdtgetd
601 idpv
```

SEQ ID NO:2

FIG. 10

Modified Dicer (Δ DEAD)

```
    mddddv fppyvlrpdd ggprvtinta ighinrycar lpsdpfthla pkcrtrelpd
gtfystlylp insplrasiv gppmscvrla ervvalicce klhkigeldd hlmpvgketv
kyeeeldlhd eeetsvpgrp gstkrrqcyp kaipeclrds yprpdqpcyl yvigmvlttp
lpdelnfrrr klyppedttr cfgiltakpi pqiphfpvyt rsgevtisie lkksgfmlsl
qmlelitrlh qyifshilrl ekpalefkpt dadsaycvlp lnvvndsstl didfkfmedi
eksearigip stkytketpf vfkledyqda viipryrnfd qphrfyvadv ytdltplskf
pspeyetfae yyktkynldl tnlnqplldv dhtssrlnll tprhlnqkgk alplssaekr
kakweslqnk qilvpelcai hpipaslwrk avclpsilyr lhclltaeel raqtasdagv
gvrslpadfr ypnldfgwkk sidsksfisi snsssaendn yckhstivpe naahqganrt
sslenhdqms vncrtllses pgklhvevsa dltainglsy nqnlangsyd lanrdfcqgn
qlnyykqeip vqpttsysiq nlysyenqpq psdectllsn kyldgnanks tsdgspvmav
mpgttdtiqv lkgrmdseqs psigyssrtl gpnpglilqa ltlsnasdgf nlerlemlgd
sflkhaitty lfctypdahe grlsymrskk vsncnlyrlg kkkglpsrmv vsifdppvnw
lppgyvvnqd ksntdkwekd emtkdcmlan gkldedyeee deeeeslmwr apkeeadyed
dfleydqehi rfidnmlmgs gafvkkisls pfsttdsaye wkmpkksslg smpfssdfed
fdysswdamc yldpskavee ddfvvgfwnp seencgvdtg kqsisydlht eqciadksia
dcveallgcy ltscgeraaq lflcslglkv lpvikrtdre kalcptrenf nsqqknlsvs
caaasvassr ssvlkdseyg clkipprcmf dhpdadktln hlisgfenfe kkinyrfknk
ayllqaftha syhyntitdc yqrleflgda ildylitkhl yedprqhspg vltdlrsalv
nntifaslav kydyhkyfka vspelfhvid dfvqfqlekn emqgmdselr rseedeekee
dievpkamgd ifeslagaiy mdsgmsletv wqvyypmmrp liekfsanvp rspvrellem
epetakfspa ertydgkvrv tvevvgkgkf kgvgrsyria ksaaarralr slkanqpqvp
ns
```

(SEQ ID NO:3)

FIG. 11

Modified Dicer (K70A)

```
   1 mkspalqpls maglqlmtpa sspmgpffgl pwqqeaihdn iytprkyqve lleaaldhnt
  61 ivclntgsga tfiavlltke lsyqirgdfs rngkrtvflv nsanqvaqqv savrthsdlk
 121 vgeysnlevn aswtkerwnq eftkhqvlim tcyvalnvlk ngylslsdin llvfdechla
 181 ildhpyreim klcencpscp rilgltasil ngkcdpeele ekiqklekil ksnaetatdl
 241 vvldrytsqp ceivvdcgpf tdrsglyerl lmeleealnf indcnisvhs kerdstlisk
 301 qilsdcravl vvlgpwcadk vagmmvrelq kyikheqeel hrkfllftdt flrkihalce
 361 ehfspasldl kfvtpkvikl leilrkykpy erqqfesvew ynnrnqdnyv swsdseddde
 421 deeieekekp etnfpspftn ilcgiifver rytavvlnrl ikeagkqdpe layissnfit
 481 ghgigknqpr nkqmeaefrk qeevlrkfra hetnlliats iveegvdipk cnlvvrfdlp
 541 teyrsyvqsk grarapisny imladtdkik sfeedlktyk aiekilrnkc sksvdtgetd
 601 idpvmddddv fppyvlrpdd ggprvtinta ighinrycar lpsdpfthla pkcrtrelpd
 661 gtfystlylp insplrasiv gppmscvrla ervvalicce klhkigeldd hlmpvgketv
 721 kyeeeldlhd eeetsvpgrp gstkrrqcyp kaipeclrds yprpdqpcyl yvigmvlttp
 781 lpdelnfrrr klyppedttr cfgiltakpi pqiphfpvyt rsgevtisie lkksgfmlsl
 841 qmlelitrlh qyifshilrl ekpalefkpt dadsaycvlp lnvvndsstl didfkfmedi
 901 eksearigip stkytketpf vfkledyqda viipryrnfd qphrfyvadv ytdltplskf
 961 pspeyetfae yyktkynldl tnlnqplldv dhtssrlnll tprhlnqkgk alplssaekr
1021 kakweslqnk qilvpelcai hpipaslwrk avclpsilyr lhclltaeel raqtasdagv
1081 gvrslpadfr ypnldfgwkk sidsksfisi snsssaendn yckhstivpe naahqganrt
1141 sslenhdqms vncrtllses pgklhvevsa dltainglsy nqnlangsyd lanrdfcqgn
1201 qlnyykqeip vqpttsysiq nlysyenqpq psdectllsn kyldgnanks tsdgspvmav
1261 mpgttdtiqv lkgrmdseqs psigyssrtl gpnpglilqa ltlsnasdgf nlerlemlgd
1321 sflkhaitty lfctypdahe grlsymrskk vsncnlyrlg kkkglpsrmv vsifdppvnw
1381 lppgyvvnqd ksntdkwekd emtkdcmlan gkldedyeee deeeeslmwr apkeeadyed
1441 dfleydqehi rfidnmlmgs gafvkkisls pfsttdsaye wkmpkksslg smpfssdfed
1501 fdysswdamc yldpskavee ddfvvgfwnp seencgvdtg kqsisydlht eqciadksia
1561 dcveallgcy ltscgeraaq lflcslglkv lpvikrtdre kalcptrenf nsqqknlsvs
1621 caaasvassr ssvlkdseyg clkipprcmf dhpdadktln hlisgfenfe kkinyrfknk
1681 ayllqaftha syhyntitdc yqrleflgda ildylitkhl yedprqhspg vltdlrsalv
1741 nntifaslav kydyhkyfka vspelfhvid dfvqfqlekn emqgmdselr rseedeekee
1801 dievpkamgd ifeslagaiy mdsgmsletv wqvyypmmrp liekfsanvp rspvrellem
1861 epetakfspa ertydgkvrv tvevvgkgkf kgvgrsyria ksaaarralr slkanqpqvp
1921 ns
```

(SEQ ID NO:4)

FIG. 12

Dicer amino acid sequence alignment

```
Sequence 1 - Homo sapiens -- GenBank NP_803187
Sequence 2 - Pan troglodytes -- GenBank XP_001154010
Sequence 3 - Canis familiaris -- GenBank XP_537547
Sequence 4 - Rattus norvegicus -- GenBank XP_001068155
Sequence 5 - Mus musculus -- GenBank EDL18787

sequence1       MKSPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNT 60
sequence2       MKNPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNT 60
sequence3       MKSPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNT 60
sequence4       MKSPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNT 60
sequence5       LKSPALQPLSMAGLQLMTPASSPMGPFFGLPWQQEAIHDNIYTPRKYQVELLEAALDHNT 60
                :*.*********************************************************

sequence1       IVCLNTGSGKTFIAVLLTKELSYQIRGDFSRNGKRTVFLVNSANQVAQQVSAVRTHSDLK 120
sequence2       IVCLNTGSGKTFIAVLLTKELSYQIRGDFSRNGKRTVFLVNSANQVAQQVSAVRTHSDLK 120
sequence3       IVCLNTGSGKTFIAVLLTKELSYQIRGDFNRNGKRTVFLVNSANQVAQQVSAVRTHSDLK 120
sequence4       IVCLNTGSGKTFIAVLLTKELAHQIRGDLSPHAKRTVFLVNSANQVAQQVSAVRTHSDLK 120
sequence5       IVCLNTGSGKTFIAVLLTKELAHQIRGDLNPHAKRTVFLVNSANQVAQQVSAVRTHSDLK 120
                *********************::*****:.  :.**************************

sequence1       VGEYSNLEVNASWTKERWNQEFTKHQVLIMTCYVALNVLKNGYLSLSDINLLVFDECHLA 180
sequence2       VGEYSNLEVNASWTKERWNQEFTKHQVLIMTCYVALNVLKNGYLSLSDINLLVFDECHLA 180
sequence3       VGEYSNLEVNASWTKEKWNQEFTKHQVLVMTCYVALNVLKNGYLSLSDINLLVFDECHLA 180
sequence4       VGEYSNLEVNASWTKERWSQEFTKHQVLIMTCYVALNVLKNGYLSLSDINLLVFDECHLA 180
sequence5       VGEYSDLEVNASWTKERWSQEFTKHQVLIMTCYVALTVLKNGYLSLSDINLLVFDECHLA 180
                *****:*********:*.*********:*******.************************
```

FIG. 13A

```
sequence1     ILDHPYREIMKLCENCPSCPRILGLTASILNGKCDPEELEEKIQKLEKILKSNAETATDL 240
sequence2     ILDHPYREIMKLCENCPSCPRILGLTASILNGKCDPEELEEKIQKLEKILKSNAETATDL 240
sequence3     ILDHPYREIMKLCENCPSCPRILGLTASILNGKCDPEELEEKIQKLEKILKSNAETATDL 240
sequence4     ILDHPYREIMKLCDSCPSCPRILGLTASILNGKCDPDELEEKIQKLEKILKSGAETATDL 240
sequence5     ILDHPYREIMKLCESCPSCPRILGLTASILNGKCDPEELEEKIQKLERILRSDAETATDL 240
              *************:.**********************:*********:**:*.*******

sequence1     VVLDRYTSQPCEIVVDCGPFTDRSGLYERLLMELEEALNFINDCNISVHSKERDSTLISK 300
sequence2     VVLDRYTSQPCEIVVDCGPFTDRSGLYERLLMELEEALNFINDCNISVHSKERDSTLISK 300
sequence3     VVLDRYTSQPCEIVVDCGPFTDRSGLYGRLLVELEEALNFINDCNISVHSKERDSTLISK 300
sequence4     VVLDRYTSQPCEIVVDCGPFTDRSGLYERLLMELEEALDFINDCNVSVHSKERDSTLISK 300
sequence5     VVLDRYTSQPCEIVVDCGPFTDRSGLYERLLMELEAALDFINDCNVAVHSKERDSTLISK 300
              *************************** ***:*** **:******::*************

sequence1     QILSDCRAVLVVLGPWCADKVAGMMVRELQKYIKHEQEELHRKFLLFTDTFLRKIHALCE 360
sequence2     QILSDCRAVLVVLGPWCADKVAGMMVRELQKYIKHEQEELHRKFLLFTDTFLRKIHALCE 360
sequence3     QILSDCRAVLVVLGPWCADKVAGMMVRELQKYIKHEQEELHRKFLLFTDTFLRKIHALCE 360
sequence4     QILSDCRAVLVVLGPWCADKVAGMMVRELQKYIKHEQEELHRKFLLFTDTLLRKIHALCE 360
sequence5     QILSDCRAVLVVLGPWCADKVAGMMVRELQKYIKHEQEELHRKFLLFTDTLLRKIHALCE 360
              **************************************************:*********

sequence1     EHFSPASLDLKFVTPKVIKLLEILRKYKPYERQQFESVEWYNNRNQDNYVSWSDSEDDDE 420
sequence2     EHFSPASLDLKFVTPKVIKLLEILRKYKPYERQQFESVEWYNNRNQDNYVSWSDSEDDDE 420
sequence3     EHFSPASLDLKFVTPKVIKLLEILRKYKPYERQQFESVEWYNNRNQDNYVSWSDSEDDDE 420
sequence4     EYFSPASLDLKYVTPKVMKLLEILRKYKPYERQQFESVEWYNNRNQDNYVSWSDSEDDDD 420
sequence5     EYFSPASLDLKYVTPKVMKLLEILRKYKPYERQQFESVEWYNNRNQDNYVSWSDSEDDDD 420
              *:*********:*****:*****************************************:
```

FIG. 13B

```
sequence1    DEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT 480
sequence2    DEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT 480
sequence3    DEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT 480
sequence4    DEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT 480
sequence5    DEEIEEKEKPETNFPSPFTNILCGIIFVERRYTAVVLNRLIKEAGKQDPELAYISSNFIT 480
             ************************************************************

sequence1    GHGIGKNQPRNKQMEAEFRKQEEVLRKFRAHETNLLIATSIVEEGVDIPKCNLVVRFDLP 540
sequence2    GHGIGKNQPRNKQMEAEFRKQEEVLRKFRAHETNLLIATSIVEEGVDIPKCNLVVRFDLP 540
sequence3    GHGIGKNQPRNKQMEAEFRKQEEVLRKFRAHETNLLIATSIVEEGVDIPKCNLVVRFDLP 540
sequence4    GHGIGKNQPRSKQMEAEFRKQEEVLRKFRAHETNLLIATSVVEEGVDIPKCNLVVRFDLP 540
sequence5    GHGIGKNQPRSKQMEAEFRKQEEVLRKFRAHETNLLIATSVVEEGVDIPKCNLVVRFDLP 540
             **********.*****************************:*******************

sequence1    TEYRSYVQSKGRARAPISNYIMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSVDTGETD 600
sequence2    TEYRSYVQSKGRARAPISNYIMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSVDTGEID 600
sequence3    TEYRSYVQSKGRARAPISNYIMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSVDTGETD 600
sequence4    TEYRSYVQSKGRARAPISNYVMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSVDGAEAD 600
sequence5    TEYRSYVQSKGRARAPISNYVMLADTDKIKSFEEDLKTYKAIEKILRNKCSKSADGAEAD 600
             ********************:********************************.* .* *

sequence1    IDPVMDDDDVFPPYVLRPDDGGPRVTINTAIGHINRYCARLPSDPFTHLAPKCRTRELPD 660
sequence2    IDPVMDDDDVFPPYVLRPDDGGPRVTINTAIGHINRYCARLPSDPFTHLAPKCRTRELPD 660
sequence3    IEPVVDDDDVFPPYVLRPDDGGPRVTINTAIGHINRYCARLPSDPFTHLAPKCRTRELPD 660
sequence4    VHAVVDDDDVFPPYVLRPDDGGPRVTINTAIGHINRYCARLPSDPFTHLAPKCRTRELPD 660
sequence5    VHAGVDDEDAFPPYVLRPDDGGPRVTINTAIGHINRYCARLPSDPFTHLAPKCRTRELPD 660
             :.. :**:*.**************************************************
```

FIG. 13C

```
sequence1      GTFYSTLYLPINSPLRASIVGPPMSCVRLAERVVALICCEKLHKIGELDDHLMPVGKETV 720
sequence2      GTFYSTLYLPINSPLRASIVGPPMSCVRLAERVVALICCEKLHKIGELDDHLMPVGKETV 720
sequence3      GTFYSTLYLPINSPLRASIVGPPMSCVRLAERVVALICCEKLHKIGELDDHLMPVGKETV 720
sequence4      GTFYSTLYLPINSPLRASIVGPPMGCVRLAERVVALICCEKLHKIGELDEHLMPVGKETV 720
sequence5      GTFYSTLYLPINSPLRASIVGPPMDSVRLAERVVALICCEKLHKIGELDEHLMPVGKETV 720
               ************************..***********************:**********

sequence1      KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRDSYPRPDQPCYLYVIGMVLTTP 780
sequence2      KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRDSYPRPDQPCYLYVIGMVLTTP 780
sequence3      KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRDSYPKPDQPCYLYVIGMVLTTP 780
sequence4      KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRESYPKPDQPCYLYVIGMVLTTP 780
sequence5      KYEEELDLHDEEETSVPGRPGSTKRRQCYPKAIPECLRESYPKPDQPCYLYVIGMVLTTP 780
               **************************************:***:*****************

sequence1      LPDELNFRRRKLYPPEDTTRCFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFMLSL 840
sequence2      LPDELNFRRRKLYPPEDTTRCFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFMLSL 840
sequence3      LPDELNFRRRKLYPPEDTTRCFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFTLSL 840
sequence4      LPDELNFRRRKLYPPEDTTRCFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFTLSQ 840
sequence5      LPDELNFRRRKLYPPEDTTRCFGILTAKPIPQIPHFPVYTRSGEVTISIELKKSGFTLSQ 840
               ******************************************************** ** 

sequence1      QMLELITRLHQYIFSHILRLEKPALEFKPTDADSAYCVLPLNVVNDSSTLDIDFKFMEDI 900
sequence2      QMLELITRLHQYIFSHILRLEKPALEFKPTDADSAYCVLPLNVVNDSSTLDIDFKFMEDI 900
sequence3      QMLELITRLHQYIFSHILRLEKPALEFKPTDADSAYCVLPLNVVNDSSTLDIDFKFMEDI 900
sequence4      QMLELVTRLHQYIFSHILRLEKPALEFQPAGAESAYCVLPLNVVNDSSTLDIDFKFMEDI 900
sequence5      QMLELITRLHQYIFSHILRLEKPALEFKPTGAESAYCVLPLNVVNDSGTLDIDFKFMEDI 900
               *****:*********************:*:.*:**************.************
```

FIG. 13D

```
sequence1     EKSEARIGIPSTKYTKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF 960
sequence2     EKSEARIGIPSTKYTKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF 960
sequence3     EKSEARIGIPSTKYSKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF 960
sequence4     EKSEARIGIPSTKYSKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF 960
sequence5     EKSEARIGIPSTKYSKETPFVFKLEDYQDAVIIPRYRNFDQPHRFYVADVYTDLTPLSKF 960
              **************:*********************************************

sequence1     PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKR 1020
sequence2     PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKR 1020
sequence3     PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKR 1020
sequence4     PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKR 1020
sequence5     PSPEYETFAEYYKTKYNLDLTNLNQPLLDVDHTSSRLNLLTPRHLNQKGKALPLSSAEKR 1020
              ************************************************************

sequence1     KAKWESLQNKQILVPELCAIHPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGV 1080
sequence2     KAKWESLQNKQILVPELCAIHPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGV 1080
sequence3     KAKWESLQNKQILVPELCAIHPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGV 1080
sequence4     KAKWESLQNKQILVPELCAIHPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGV 1080
sequence5     KAKWESLQNKQILVPELCAIHPIPASLWRKAVCLPSILYRLHCLLTAEELRAQTASDAGV 1080
              ************************************************************

sequence1     GVRSLPADFRYPNLDFGWKKSIDSKSFISISNSSSAENDNYCKHSTIVP-ENAAHQGANR 1139
sequence2     GVRSLPADFRYPNLDFGWKKSIDSKSFISISNSSSAENDNYCKHSTIVP-ENAAHQGANR 1139
sequence3     GVRSLPVDFRYPNLDFGWKKSIDSKSFISVANSSSAENENYCKHSTIVVPENAARQGANR 1140
sequence4     GVRSLPADFRYPNLDFGWKKSIDSKSFISTCNSSLAESDNYCKHSTTVVPENAAHQGATR 1140
sequence5     GVRSLPVDFRYPNLDFGWKKSIDSKSFISSCNSSLAESDNYCKHSTTVVPEHAAHQGATR 1140
              ******.**********************  .*** **.:******* *  *:**:***.*
```

FIG. 13E

```
sequence1      TSSLENHDQMSVNCRTLLSESPGKLHVEVSADLTAINGLSYNQNLANGSYDLANRDFCQG 1199
sequence2      TSSLENHDQMSVNCRTLLSESPGKLHVEVSADLTAINGLSYNQNLANGSYDLANRDFCQG 1199
sequence3      TSSLENHDQMSVNCRTLFSESPGKLQIEVVTDLTAINGLSYNKNLANGSYDLANRDFCQG 1200
sequence4      P-SLENHDQMSVNCKRLPAESPAKLQSEVSVDLTAINGLSYNKSLANGSYDLVNRDFCQG 1199
sequence5      P-SLENHDQMSVNCKRLPAESPAKLQSEVSTDLTAINGLSYNKNLANGSYDLVNRDFCQG 1199
               . ************: * :***.**: ** .***********:.********.*******

sequence1      NQLNYYKQEIPVQPTTSYSIQNLYSYENQPQPSDECTLLSNKYLDGNANKSTSDGSPVMA 1259
sequence2      NQLNYYKQEIPVQPTTSYSIQNLYSYENQPQPSDECTLLSNKYLDGNANKSTSDGSPVMA 1259
sequence3      NQLNYYKQEIPVQPTTSYPIQNLYNYENQPKPSDECTLLSNKYLDGNANKSTSDGSPTTA 1260
sequence4      NQLTYFKQEIPVQPTTSYPIQNLYNYENQPTPSNECPLLSNKYLDGNANTSTSDGSPAGS 1259
sequence5      NQLNYFKQEIPVQPTTSYPIQNLYNYENQPKPSNECPLLSNTYLDGNANTSTSDGSPAVS 1259
               ***.*:************.*****.***** **:**.****.*******.*******. :

sequence1      VMPGTTDTIQVLKGRMDSEQSPSIGYSSRTLGPNPGLILQALTLSNASDGFNLERLEMLG 1319
sequence2      VMPGTTDTIQVLKGRMDSEQSPSIGYSSRTLGPNPGLILQALTLSNASDGFNLERLEMLG 1319
sequence3      AMPGTTEAVRALKDKMGSEQSPCPGYSSRTLGPNPGLILQALTLSNASDGFNLERLEMLG 1320
sequence4      PRPAMMTAVEALEGRTDSEQSPSVGHSSRTLGPNPGLILQALTLSNASDGFNLERLEMLG 1319
sequence5      TMPAMMNAVKALKDRMDSEQSPSVGYSSRTLGPNPGLILQALTLSNASDGFNLERLEMLG 1319
                 *.    ::..*:.: .*****. *:*********************************

sequence1      DSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLGKKKGLPSRMVVSIFDPPVN 1379
sequence2      DSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLGKKKGLPSRMVVSIFDPPVN 1379
sequence3      DSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLGKKKGLPSRMVVSIFDPPVN 1380
sequence4      DSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLGKKQGLPSRMVVSIFDPPVN 1379
sequence5      DSFLKHAITTYLFCTYPDAHEGRLSYMRSKKVSNCNLYRLGKKKGLPSRMVVSIFDPPVN 1379
               *******************************************:****************
```

FIG. 13F

```
sequence1     WLPPGYVVNQDKSNTDKWEKDEMTKDCMLANGKLDEDYEEEDEEEESLMWRAPKEEADYE 1439
sequence2     WLPPGYVVNQDKSNTDKWEKDEMTKDCMLANGKLDEDYEEEDEEEESLMWRAPKEEADYE 1439
sequence3     WLPPGYVVNQDKSNADKWEKDEMTKDCMLANGKLDEDFEEDDEEEEDLMWRAPKEDADYE 1440
sequence4     WLPPGYVVNQDKSNSEKWEKDEMTKDCLLANGKLGEDCE--EEEEEELAWRAPKEEAEYE 1437
sequence5     WLPPGYVVNQDKSNSEKWEKDEMTKDCLLANGKLGEACE--EEE--DLTWRAPKEEAEDE 1435
              **************::***********:******.*  *  :**  .* ******:*: *

sequence1     DDFLEYDQEHIRFIDNMLMGSGAFVKKISLSPFSTTDSAYEWKMPKKSSLGSMPFSSDFE 1499
sequence2     DDFLEYDQEHIRFIDNMLMGSGAFVKKISLSPFSTTDSAYEWKMPKKSSLGSMPFSSDFE 1499
sequence3     DDFLEYDQEHIKFIDNMLMGSGAFVKKISLSPFSTTDSAYEWKMPKKSSLGSMPFSSDFE 1500
sequence4     DDLLEYDQEHIQFIDSMLMGSGAFVKKIPLSPFSTSDSAYEWKMPKKASLGSVPFSSDLE 1497
sequence5     DDFLEYDQEHIQFIDSMLMGSGAFVRKISLSPFSASDSAYEWKMPKKASLGSMPFASGLE 1495
              **:********:***.*********:**.*****::***********:****:**:*.:*

sequence1     DFDYSSWDAMCYLDPSKAVEEDDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSI 1559
sequence2     DFDYSSWDAMCYLDPSKAVEEDDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSI 1559
sequence3     DFDYSSWDAMCYLDPSKAVEEDDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSI 1560
sequence4     DFDYSSWDAMCYLDPSKAVEEDDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSI 1557
sequence5     DFDYSSWDAMCYLDPSKAVEEDDFVVGFWNPSEENCGVDTGKQSISYDLHTEQCIADKSI 1555
              ************************************************************

sequence1     ADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVIKRTDREKALCPTRENFNSQQKNLSV 1619
sequence2     ADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVIKRTDREKALCPTRENFNSQQKNLSV 1619
sequence3     ADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVMKRTDREKTMCPPRENFSSQQKNLSG 1620
sequence4     ADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVIKRTSRDKASYPAQENSSSQQKSPSG 1617
sequence5     ADCVEALLGCYLTSCGERAAQLFLCSLGLKVLPVIKRTSREKALDPAQENGSSQQKSLSG 1615
              **********************************:***.*:*:  *.:** .****. *
```

FIG. 13G

```
sequence1      SCAAASVASSRSSVLKDSEYGCLKIPPRCMFDHPDADKTLNHLISGFENFEKKINYRFKN 1679
sequence2      SCAAASVASSRSSVLKDSEYGCLKIPPRCMFDHPDADKTLNHLISGFENFEKKINYRFKN 1679
sequence3      GRAAASVASLRPSVLKDLEYGCLKIPPRCMFDHPDADKTLNHLISGFENFEKKINYRFKN 1680
sequence4      SCAAA--VSPRSSAGKDLEYGCLKIPPRCMFDHPDAEKTLNHLISGFENFEKKINYIFKN 1675
sequence5      SCASP--VGPRSSAGKDLEYGCLKIPPRCMFDHPDAEKTLNHLISGFETFEKKINYRFKN 1673
               . *:.  .. *.*. ** ******************:***********.******* ***

sequence1      KAYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSAL 1739
sequence2      KAYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSAL 1739
sequence3      KAYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSAL 1740
sequence4      KAYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSAL 1735
sequence5      KAYLLQAFTHASYHYNTITDCYQRLEFLGDAILDYLITKHLYEDPRQHSPGVLTDLRSAL 1733
               ************************************************************

sequence1      VNNTIFASLAVKYDYHKYFKAVSPELFHVIDDFVQFQLEKNEMQGMDSELRRSEEDEEKE 1799
sequence2      VNNTIFASLAVKYDYHKYFKAVSPELFHVIDDFVQFQLEKNEMQGMDSELRRSEEDEEKE 1799
sequence3      VNNTIFASLAVKYDYHKYFKAVSPELFHVIDDFVQFQLEKNEMQGMDSELRRSEEDEEKE 1800
sequence4      VNNTIFASLAVKYDYHKYFKAVSPELFHVIDDFVQFQLEKNEMQGMDSELRRSEEDEEKE 1795
sequence5      VNNTIFASLAVKYDYHKYFKAVSPELFHVIDDFVKFQLEKNEMQGMDSELRRSEEDEEKE 1793
               **********************************:*************************

sequence1      EDIEVPKAMGDIFESLAGAIYMDSGMSLETVWQVYYPMMRPLIEKFSANVPRSPVRELLE 1859
sequence2      EDIEVPKAMGDIFESLAGAIYMDSGMSLETVWQVYYPMMRPLIEKFSANVPRSPVRELLE 1859
sequence3      EDIEVPKAMGDIFESLAGAIYMDSGMSLEMVWQVYYPMMRPLIEKFSANVPRSPVRELLE 1860
sequence4      EDIEVPKAMGDIFESLAGAIYMDSGMSLEVVWQVYYPMMRPLIEKFSANVPRSPVRELLE 1855
sequence5      EDIEVPKAMGDIFESLAGAIYMDSGMSLEVVWQVYYPMMQPLIEKFSANVPRSPVRELLE 1853
               ***************************** ********:*********************
```

FIG. 13H

```
sequence1        MEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPQV 1919
sequence2        MEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPQL 1919
sequence3        MEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPQV 1920
sequence4        MEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPLV 1915
sequence5        MEPETAKFSPAERTYDGKVRVTVEVVGKGKFKGVGRSYRIAKSAAARRALRSLKANQPQV 1913
                 ********************************************************** :

sequence1        PNS-------- 1922
sequence2        WVSLALPSTYQ 1930
sequence3        PNS-------- 1923
sequence4        PNS-------- 1918
sequence5        PNS-------- 1916
                   *
```

FIG. 13I

METHOD OF PRODUCING DICER

CROSS-REFERENCE

This application is a divisional of U.S. patent application Ser. No. 13/565,453, filed on Aug. 2, 2012, which claims the benefit of U.S. Provisional Patent Application Nos. 61/515,135, filed Aug. 4, 2011, and 61/515,647, filed Aug. 5, 2011, the disclosures of each of which applications are incorporated herein by reference in their entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

This invention was made with government support under Grant No. R01 GM073794-05 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND

RNA interference (RNAi) and related pathways trigger post-transcriptional gene silencing using single-stranded guide RNAs that base pair with cognate mRNAs to direct their endonucleolytic cleavage or translational repression by RNA-induced silencing complexes (RISCs). Silencing is initiated by long dsRNAs or RNA hairpins, which are processed by the endonuclease Dicer to yield 21-23 nt short interfering RNAs (siRNAs) or microRNAs (miRNAs), respectively. These small interfering dsRNAs are then loaded onto Argonaute2 (Ago2), the endonuclease component of RISC.

The eukaryotic endoribonuclease Dicer recognizes distinct types of double-stranded RNA (dsRNA) substrates and generates ~21 base pair products that assemble into RISCs. In humans, Dicer plays a central role in producing most of the small regulatory RNAs that enter this pathway in the cytoplasm. Structural analysis of *Giardia* Dicer and biochemical studies of human Dicer (hDicer) suggest that the enzyme functions as a monomer to bind, orient and cleave dsRNA substrates using a two-metal-ion mechanism similar to that of bacterial Ribonuclease III.

Although mammalian Dicer has been successfully produced recombinantly in eukaryotic cells, recombinant production of mammalian Dicer in prokaryotic cells has proved challenging.

LITERATURE

US Patent Publication No. 2011/0117610; U.S. Patent Publication No. 2007/0031417; U.S. Patent Publication No. 2003/0224432; WO 03/093430; MacRae and Doudna (2007) *Curr. Opin. Struct. Biol.* 17:138.

SUMMARY

The present disclosure provides a method for producing a Dicer polypeptide in a prokaryotic host cell. The present disclosure further provides a purified Dicer complex. The present disclosure further provides kits for producing a Dicer polypeptide in a prokaryotic host cell.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGS. **1**A-C depict interaction of functional fragments of human Dicer (hDcr).

FIGS. **2**A-C depict the effect of cooperative action between the PAZ and RNase III domains of hDcr on the size of dicing products.

FIGS. **3**A and **3**B depict requirement of the C-terminal dsRBD for RNA binding and cleavage in the absence of the PAZ domain.

FIGS. **4**A-C depict interaction of an active, bacterially expressed helicase fragment of Dicer with Trans-activation-responsive RNA-binding protein (TRBP).

FIGS. **5**A and **5**B depict the effect of interaction ATPase/helicase domain with hairpin loop on the substrate selection of human Dicer. Pre-hlet7a-1: SEQ ID NO:9; hlet7-stem upper strand (5' strand): SEQ ID NO:10; hlet7-stem lower strand (3' strand): SEQ ID NO:11; 37ab upper strand (5' strand): SEQ ID NO:10; 37ab lower strand (3' strand): SEQ ID NO:12; 37ab-loop: SEQ ID NO:13. FIG. **5**C depicts interaction of terminal loop with ATPase/helicase domain determines processing activity of hDcr.

FIG. **6** depicts the activity of hDcr-N/C complex expressed in trans and the activity of wild-type hDcr.

FIG. **7** depicts stable complex formation between DP and hDcr C.

FIG. **8** depicts ATPase activity of FL-hDcr and MBP-ATPase/hel.

FIG. **9** depicts the amino acid sequence of a wild-type human Dicer polypeptide (SEQ ID NO:1).

FIG. **10** depicts an amino acid sequence of a DExD/H-box domain (SEQ ID NO:2).

FIG. **11** depicts the amino acid sequence of a Dicer polypeptide that lacks a DExD/H-box domain (SEQ ID NO:3).

FIG. **12** depicts the amino acid sequence of a Dicer polypeptide that has a single amino acid substitution in the DExD/H-box domain (SEQ ID NO:4).

FIGS. **13**A-I depict an amino acid sequence alignment of Dicer polypeptides from various mammalian species. Sequence 1: SEQ ID NO:1; Sequence 2: SEQ ID NO:5; Sequence 3: SEQ ID NO:6; Sequence 4: SEQ ID NO:7; Sequence 5: SEQ ID NO:8.

DEFINITIONS

The terms "polynucleotide" and "nucleic acid," used interchangeably herein, refer to a polymeric form of nucleotides of any length, either ribonucleotides or deoxynucleotides. Thus, this term includes, but is not limited to, single-, double-, or multi-stranded DNA or RNA, genomic DNA, cDNA, DNA-RNA hybrids, or a polymer comprising purine and pyrimidine bases or other natural, chemically or biochemically modified, non-natural, or derivatized nucleotide bases. The terms "polynucleotide" and "nucleic acid" should be understood to include, as applicable to the embodiment being described, single-stranded (such as sense or antisense) and double-stranded polynucleotides.

The terms "peptide," "polypeptide," and "protein" are used interchangeably herein, and refer to a polymeric form of amino acids of any length, which can include coded and non-coded amino acids, chemically or biochemically modified or derivatized amino acids, and polypeptides having modified peptide backbones.

A "protein coding sequence" or a sequence that "encodes" a particular polypeptide or peptide, is a nucleic acid sequence that is transcribed (in the case of DNA) and is translated (in the case of mRNA) into a polypeptide in vitro or in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' terminus and a translation stop codon at the 3' terminus. A coding sequence can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or eukaryotic DNA, and synthetic nucleic acids. A transcription termination sequence will usually be located 3' to the coding sequence.

A "small interfering" or "short interfering RNA" or siRNA is a RNA duplex of nucleotides that is targeted to a gene interest (a "target gene"). An "RNA duplex" refers to the structure formed by the complementary pairing between two regions of a RNA molecule or between two separate RNA molecules. siRNA is "targeted" to a gene in that the nucleotide sequence of the duplex portion of the siRNA is complementary to a nucleotide sequence of the targeted gene. In some embodiments, the length of the duplex of siRNAs is less than 30 nucleotides. In some embodiments, the duplex can be 29, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11 or 10 nucleotides in length. In some embodiments, the length of the duplex is 19-25 nucleotides in length. The RNA duplex portion of the siRNA can be part of a hairpin structure. In addition to the duplex portion, the hairpin structure may contain a loop portion positioned between the two sequences that form the duplex. The loop can vary in length. In some embodiments the loop is 5, 6, 7, 8, 9, 10, 11, 12 or 13 nucleotides in length. The hairpin structure can also contain 3' or 5' overhang portions. In some embodiments, the overhang is a 3' or a 5' overhang 0, 1, 2, 3, 4 or 5 nucleotides in length.

MicroRNAs (miRNAs) are encoded by genes, which encode transcripts containing short double-stranded RNA hairpins. MiRNAs are transcribed as longer precursors, termed pre-miRNAs, which can be 50 to 80 nucleotides in length, and which are sometimes found in clusters and frequently found in introns. Upon transcription, miRNAs undergo nuclear cleavage by an RNase III endonuclease, producing the 60-70-nt stem-loop precursor miRNA (pre-miRNA) with a 5' phosphate and a 2-nt 3 overhang. The pre-miRNAs are cleaved by Dicer about two helical turns away from the ends of the pre-miRNA stem loop, producing double-stranded RNA with strands that are approximately the same length (21 to 24 nucleotides), and possess the characteristic 5'-phosphate and 3'-hydroxyl termini. One of the strands of this short-lived intermediate accumulates as the mature miRNA and is subsequently incorporated into a ribonucleoprotein complex, the miRNP. MiRNAs interact with target RNAs at specific sites to induce cleavage of the message or inhibit translation.

The term "naturally-occurring" as used herein as applied to a nucleic acid, a cell, or an organism, refers to a nucleic acid, cell, or organism that is found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by a human in the laboratory is naturally occurring.

As used herein the term "isolated" is meant to describe a polynucleotide, a polypeptide, or a cell that is in an environment different from that in which the polynucleotide, the polypeptide, or the cell naturally occurs. An isolated genetically modified host cell may be present in a mixed population of genetically modified host cells.

As used herein, the term "exogenous nucleic acid" refers to a nucleic acid that is not normally or naturally found in and/or produced by a given bacterium, organism, or cell in nature. As used herein, the term "endogenous nucleic acid" refers to a nucleic acid that is normally found in and/or produced by a given bacterium, organism, or cell in nature. An "endogenous nucleic acid" is also referred to as a "native nucleic acid" or a nucleic acid that is "native" to a given bacterium, organism, or cell.

The term "heterologous," as used herein in the context of a genetically modified host cell, refers to a polypeptide wherein at least one of the following is true: (a) the polypeptide is foreign ("exogenous") to (i.e., not naturally found in) the host cell; (b) the polypeptide is naturally found in (e.g., is "endogenous to") a given host microorganism or host cell but is either produced in an unnatural (e.g., greater than expected or greater than naturally found) amount in the cell, or differs in nucleotide sequence from the endogenous nucleotide sequence such that the same encoded protein (having the same or substantially the same amino acid sequence) as found endogenously is produced in an unnatural (e.g., greater than expected or greater than naturally found) amount in the cell.

The term "heterologous," as used herein in the context of a chimeric polypeptide, refers to two components that are defined by structures derived from different sources. For example, where "heterologous" is used in the context of a chimeric polypeptide (e.g., a chimeric Dicer enzyme), the chimeric polypeptide includes operably linked amino acid sequences that can be derived from different polypeptides (e.g., a first amino acid sequence from Dicer enzyme; and a second amino acid sequence other than a Dicer enzyme). Similarly, "heterologous" in the context of a polynucleotide encoding a chimeric polypeptide includes operably linked nucleotide sequences that can be derived from different coding regions (e.g., a first nucleotide sequence encoding a Dicer enzyme; and a second nucleotide sequence encoding a polypeptide other than a Dicer enzyme).

"Recombinant," as used herein, means that a particular nucleic acid (DNA or RNA) is the product of various combinations of cloning, restriction, and/or ligation steps resulting in a construct having a structural coding or non-coding sequence distinguishable from endogenous nucleic acids found in natural systems. Generally, DNA sequences encoding the structural coding sequence can be assembled from cDNA fragments and short oligonucleotide linkers, or from a series of synthetic oligonucleotides, to provide a synthetic nucleic acid which is capable of being expressed from a recombinant transcriptional unit contained in a cell or in a cell-free transcription and translation system. Such sequences can be provided in the form of an open reading frame uninterrupted by internal non-translated sequences, or introns, which are typically present in eukaryotic genes. Genomic DNA comprising the relevant sequences can also be used in the formation of a recombinant gene or transcriptional unit. Sequences of non-translated DNA may be present 5' or 3' from the open reading frame, where such sequences do not interfere with manipulation or expression of the coding regions, and may indeed act to modulate production of a desired product by various mechanisms (see "DNA regulatory sequences", below).

Thus, e.g., the term "recombinant" polynucleotide or "recombinant" nucleic acid refers to one which is not naturally occurring, e.g., is made by the artificial combination of two otherwise separated segments of sequence through human intervention. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques.

Similarly, the term "recombinant" polypeptide refers to a polypeptide which is not naturally occurring, e.g., is made by the artificial combination of two otherwise separated segments of amino sequence through human intervention. Thus, e.g., a polypeptide that comprises a heterologous amino acid sequence is recombinant.

By "construct" or "vector" is meant a recombinant nucleic acid, generally recombinant DNA, which has been generated for the purpose of the expression and/or propagation of a specific nucleotide sequence(s), or is to be used in the construction of other recombinant nucleotide sequences.

The terms "DNA regulatory sequences," "control elements," and "regulatory elements," used interchangeably herein, refer to transcriptional and translational control sequences, such as promoters, enhancers, polyadenylation signals, terminators, protein degradation signals, and the like, that provide for and/or regulate expression of a coding sequence and/or production of an encoded polypeptide in a host cell.

The term "transformation" is used interchangeably herein with "genetic modification" and refers to a permanent or transient genetic change induced in a cell following introduction of new nucleic acid (i.e., DNA exogenous to the cell). Genetic change ("modification") can be accomplished either by incorporation of the new DNA into the genome of the host cell, or by transient or stable maintenance of the new DNA as an episomal element. Where the cell is a eukaryotic cell, a permanent genetic change is generally achieved by introduction of the DNA into the genome of the cell. In prokaryotic cells, permanent changes can be introduced into the chromosome or via extrachromosomal elements such as plasmids and expression vectors, which may contain one or more selectable markers to aid in their maintenance in the recombinant host cell. Suitable methods of genetic modification include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like. The choice of method is generally dependent on the type of cell being transformed and the circumstances under which the transformation is taking place (i.e. in vitro, ex vivo, or in vivo). A general discussion of these methods can be found in Ausubel, et al, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995.

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression. As used herein, the terms "heterologous promoter" and "heterologous control regions" refer to promoters and other control regions that are not normally associated with a particular nucleic acid in nature. For example, a "transcriptional control region heterologous to a coding region" is a transcriptional control region that is not normally associated with the coding region in nature.

A "host cell," as used herein, denotes an in vivo or in vitro eukaryotic cell, a prokaryotic cell, or a cell from a multicellular organism (e.g., a cell line) cultured as a unicellular entity, which eukaryotic or prokaryotic cells can be, or have been, used as recipients for a nucleic acid (e.g., an expression vector that comprises a nucleotide sequence encoding one or more biosynthetic pathway gene products such as mevalonate pathway gene products), and include the progeny of the original cell which has been genetically modified by the nucleic acid. It is understood that the progeny of a single cell may not necessarily be completely identical in morphology or in genomic or total DNA complement as the original parent, due to natural, accidental, or deliberate mutation. A "recombinant host cell" (also referred to as a "genetically modified host cell") is a host cell into which has been introduced a heterologous nucleic acid, e.g., an expression vector. For example, a subject prokaryotic host cell is a genetically modified prokaryotic host cell (e.g., a bacterium), by virtue of introduction into a suitable prokaryotic host cell of a heterologous nucleic acid, e.g., an exogenous nucleic acid that is foreign to (not normally found in nature in) the prokaryotic host cell, or a recombinant nucleic acid that is not normally found in the prokaryotic host cell; and a subject eukaryotic host cell is a genetically modified eukaryotic host cell, by virtue of introduction into a suitable eukaryotic host cell of a heterologous nucleic acid, e.g., an exogenous nucleic acid that is foreign to the eukaryotic host cell, or a recombinant nucleic acid that is not normally found in the eukaryotic host cell.

The term "conservative amino acid substitution" refers to the interchangeability in proteins of amino acid residues having similar side chains. For example, a group of amino acids having aliphatic side chains consists of glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains consists of serine and threonine; a group of amino acids having amide-containing side chains consists of asparagine and glutamine; a group of amino acids having aromatic side chains consists of phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains consists of lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains consists of cysteine and methionine. Exemplary conservative amino acid substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

A polynucleotide or polypeptide has a certain percent "sequence identity" to another polynucleotide or polypeptide, meaning that, when aligned, that percentage of bases or amino acids are the same, and in the same relative position, when comparing the two sequences. Sequence similarity can be determined in a number of different manners. To determine sequence identity, sequences can be aligned using the methods and computer programs, including BLAST, available over the world wide web at ncbi.nlm.nih.gov/BLAST. See, e.g., Altschul et al. (1990), *J. Mol. Biol.* 215:403-10. Another alignment algorithm is FASTA, available in the Genetics Computing Group (GCG) package, from Madison, Wis., USA, a wholly owned subsidiary of Oxford Molecular Group, Inc. Other techniques for alignment are described in Methods in Enzymology, vol. 266: Computer Methods for Macromolecular Sequence Analysis (1996), ed. Doolittle, Academic Press, Inc., a division of Harcourt Brace & Co., San Diego, Calif., USA. Of particular interest are alignment programs that permit gaps in the sequence. The Smith-Waterman is one type of algorithm that permits gaps in sequence alignments. See *Meth. Mol. Biol.* 70: 173-187 (1997). Also, the GAP program using the Needleman and Wunsch alignment method can be utilized to align sequences. See *J. Mol. Biol.* 48: 443-453 (1970).

Before the present invention is further described, it is to be understood that this invention is not limited to particular embodiments described, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting, since the scope of the present invention will be limited only by the appended claims.

Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the invention.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a Dicer polypeptide" includes a plurality of such polypeptides and reference to "the Dicer complex" includes reference to one or more Dicer complexes and equivalents thereof known to those skilled in the art, and so forth. It is further noted that the claims may be drafted to exclude any optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as "solely," "only" and the like in connection with the recitation of claim elements, or use of a "negative" limitation.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable sub-combination. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided may be different from the actual publication dates which may need to be independently confirmed.

DETAILED DESCRIPTION

The present disclosure provides a method for producing a Dicer polypeptide in a prokaryotic host cell. The present disclosure further provides a purified Dicer complex. The present disclosure further provides kits for producing a Dicer polypeptide in a prokaryotic host cell.

Methods for Producing a Dicer Polypeptide

The present disclosure provides a method for producing a Dicer polypeptide in a prokaryotic host cell. The methods generally involve expressing a first Dicer polypeptide in a prokaryotic host cell, where the first Dicer polypeptide comprises a DUF and a PAZ domain, and either expressing a second Dicer polypeptide in the same prokaryotic host cell or in a separate prokaryotic host cell, where the second Dicer polypeptide comprises an RNAse IIIA domain, an RNase IIIb domain, and a double-stranded RNA binding domain (dsRBD), or where the second Dicer polypeptide comprises an RNAse IIIA domain, an RNAse IIIb domain, and lacks a functional dsRBD. The first Dicer polypeptide and the second Dicer polypeptide spontaneously associate to form an enzymatically active Dicer complex.

First Dicer Polypeptide

A first Dicer polypeptide comprises a DUF and a PAZ domain of a Dicer polypeptide. In some cases, a first Dicer polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1-1008, amino acids 1-1068, amino acids 605-1008, amino acids 605-1068, amino acids 886-1008, or amino acids 886-1068, of the amino acid sequence set forth in FIG. **9** (SEQ ID NO:1). The first Dicer polypeptide lacks RNAse IIIA domain, an RNase IIIb domain, and a double-stranded RNA binding domain. In some cases, the first Dicer polypeptide includes a DExD/H-box domain. In other cases, the first Dicer polypeptide lacks a DExD/H-box domain.

The first Dicer polypeptide can have a length of from about 300 amino acids (aa) to about 1300 aa, e.g., from about 300 aa to about 400 aa, from about 400 aa to about 500 aa, from about 500 aa to about 600 aa, from about 600 aa to about 700 aa, from about 700 aa to about 800 aa, from about 800 aa to about 900 aa, from about 900 aa to about 1000 aa, from about 1000 aa to about 1100 aa, from about 1100 aa to about 1200 aa, or from about 1200 aa to about 1300 aa.

In some embodiments, the first Dicer polypeptide lacks all or a portion of a DExD/H-box helicase domain, and comprises, a domain of unknown function ("DUF283") domain, and a PAZ domain. The DUF and PAZ domains are located in a fragment of amino acids 605 to 1068 of the amino acid sequence depicted in FIG. **9** (SEQ ID NO:1). See, e.g., MacRae and Doudna (2007) *Curr. Opin. Struct. Biol.* 17:138.

In some embodiments, the first Dicer polypeptide lacks all or a portion of a DExD/H-box helicase domain. The DExD/H-box helicase domain is an N-terminal domain found in many Dicer proteins, and is typically about 600 amino acids in length. In some embodiments, the first Dicer polypeptide lacks from about 200 amino acids to about 250 amino acids, from about 250 amino acids to about 300 amino acids, from about 300 amino acids to about 350 amino acids, from about 350 amino acids to about 400 amino acids, from about 400 amino acids to about 450 amino acids, from about 450 amino acids to about 500 amino acids, from about 500 amino acids to about 550 amino acids, or from about 550 amino acids to about 600 amino acids of a DExD/H-box helicase domain. An exemplary DExD/H-box amino acid sequence is depicted in FIG. **10** (SEQ ID NO:2).

In some embodiments, a first Dicer polypeptide comprises one or more amino acid substitutions, insertions, or deletions in the DExD/H-box domain (e.g., within amino acids 1 to about 604 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), where the one or more amino acid substitutions, insertions, or deletions result in enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$). In some embodiments, a first Dicer polypeptide comprises one or more amino acid substitutions, insertions, or deletions in the DExD/H-box domain (e.g., within amino acids 63 to 71 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), where the one or more amino acid substitutions, insertions, or deletions result in enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$). In some embodiments, a first Dicer polypeptide comprises one or more amino acid substitutions, insertions, or deletions in the DExD/H-box domain (e.g., within amino acids 175 to 178 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), where the one or more amino acid substitutions, insertions, or deletions result in enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$).

In some embodiments, the first modified Dicer polypeptide comprises one or more amino acid substitutions in the DExD/H-box domain (e.g., within amino acids 1 to about 604 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1, where the one or more amino acid substitutions results in enhanced enzymatic activity (e.g., one or more of increased k_{cat}, decreased K_m, and increased $k_{cat} \times K_m^{-1}$).

As one non-limiting example, in some embodiments, the first Dicer polypeptide comprises a K70A substitution in the DExD/H-box domain (e.g., within amino acids 1 to about 604 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), or a K70A substitution at a corresponding amino acid position, compared to a Dicer polypeptide from a species other than human. For example, in some embodiments, a first Dicer polypeptide: a) comprises a K70A substitution in the DExD/H-box domain, as shown in FIG. **12**; b) shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity over a contiguous stretch of from about 1600 amino acids to about 1700 amino acids, from about 1700 amino acids to about 1800 amino acids, or from about 1800 amino acids to about 1921 amino acids, of the amino acid sequence depicted in FIG. **12** and set forth in SEQ ID NO:4; and c) enhanced enzymatic activity (e.g., one or more of increased k_{cat}, decreased K_m, and increased $k_{cat} \times K_m^{-1}$) compared to a Dicer polypeptide comprising the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1.

In some embodiments, a first Dicer polypeptide comprises a K70A substitution in the DExD/H-box domain (e.g., within amino acids 1 to 604 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), and shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity over a contiguous stretch of at least about 1100 amino acids, at least about 1200 amino acids, or at least about 1300 amino acids, of amino acids 605-1922 of the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1.

As another example, a first Dicer polypeptide comprises one or more amino acid substitutions, insertions, or deletions in the DExD/H-box domain (e.g., within amino acids 63 to 71 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), where the one or more amino acid substitutions, insertions, or deletions result in enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$). For example, in some embodiments, a first Dicer polypeptide comprises one or more amino acid substitutions in the amino acid sequence CLNTGSGKT (SEQ ID NO:19) of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence of a Dicer polypeptide other than a human Dicer polypeptide. As shown in the amino acid sequence alignment presented in FIGS. **13**A-I, the amino acid sequence CLNTGSGKT (SEQ ID NO:19) is conserved among Dicer polypeptides from various mammalian species.

For example, in some embodiments, a first Dicer polypeptide comprises one or more non-conservative amino acid substitutions in the amino acid sequence CLNTGSGKT (SEQ ID NO:19) of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence of a Dicer polypeptide other than a human Dicer polypeptide. Exemplary, non-limiting examples of amino acid substitutions include, e.g., CLNDGSGKT (SEQ ID NO:20); CLNTPSGKT (SEQ ID NO:21); CLSTGSGKT (SEQ ID NO:22); and the like. For example, in some embodiments, a first Dicer polypeptide: a) comprises a non-conservative amino acid substitution in the amino acid sequence CLNTGSGKT (SEQ ID NO:19); e.g., amino acids 63-71 of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence from a Dicer polypeptide other than a human Dicer polypeptide; b) shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity over a contiguous stretch of from about 1600 amino acids to about 1700 amino acids, from about 1700 amino acids to about 1800 amino acids, or from about 1800 amino acids to about 1921 amino acids, of the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1; and c) enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$) compared to a Dicer polypeptide comprising the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1.

As another example, a first Dicer polypeptide comprises one or more amino acid substitutions, insertions, or deletions in the DExD/H-box domain (e.g., within amino acids 175-178 of the amino acid sequence depicted in FIG. **9**, and as set forth in SEQ ID NO:1), where the one or more amino acid substitutions, insertions, or deletions result in enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$). For example, in some embodiments, a first Dicer polypeptide comprises one or more amino acid substitutions in the amino acid sequence DECH (SEQ ID NO:23) of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence of a Dicer polypeptide other than a human Dicer polypeptide. As shown in the amino acid sequence alignment presented in FIGS. **13**A-I, the amino acid sequence DECH (SEQ ID NO:23) is conserved among Dicer polypeptides from various mammalian species.

For example, in some embodiments, a first Dicer polypeptide comprises one or more non-conservative amino acid substitutions in the amino acid sequence DECH (SEQ ID NO:23) of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence of a Dicer polypeptide other than a human Dicer polypeptide. For example, in some embodiments, a first Dicer polypeptide: a) comprises a non-conservative amino acid substitution in the amino acid sequence DECH (SEQ ID NO:23; e.g., amino acids 175-178 of the amino acid sequence depicted in FIG. **9**, or a corresponding amino acid sequence from a Dicer polypeptide other than a human Dicer polypeptide; b) shares at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or 100%, amino acid sequence identity over a contiguous stretch of from about 1600 amino acids to about 1700 amino acids, from about 1700 amino acids to about 1800 amino acids, or from about 1800 amino acids to about 1921 amino acids, of the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1; and c) enhanced enzymatic activity (e.g., increased k_{cat} and/or increased $k_{cat} \times K_m^{-1}$) compared to a Dicer polypeptide comprising the amino acid sequence depicted in FIG. **9** and set forth in SEQ ID NO:1.

In some embodiments, the first Dicer polypeptide is a chimeric Dicer polypeptide, e.g., the first Dicer polypeptide comprises a heterologous polypeptide. A heterologous polypeptide can be present at the carboxyl terminus, at the amino terminus, or at an internal site within the first Dicer polypeptide. Suitable heterologous polypeptides include, e.g., epitope tags, including, but not limited to, hemagglutinin, FLAG, and the like; proteins that provide for a detectable signal, including, but not limited to, fluorescent proteins, enzymes (e.g., β-galactosidase, alkaline phosphatase, luciferase, horse radish peroxidase, etc.), and the like; polypeptides that facilitate purification or isolation of the fusion protein, e.g., metal ion binding polypeptides such as 6 His tags, glutathione-S-transferase; etc.

Second Dicer Polypeptide

In some embodiments, the second Dicer polypeptide comprises an RNAse IIIA domain, an RNAse IIIb domain, and a double-stranded RNA binding domain (dsRBD), where such domains are included in a fragment of from about amino acid 1235 to 1922 of the amino acid sequence depicted in FIG. **9**. See, e.g., MacRae and Doudna (2007) *Curr. Opin. Struct. Biol.* 17:138. The second Dicer polypeptide lacks a DUF domain, a PAZ domain, and a DExD/H-box domain.

In other embodiments, the second Dicer polypeptide comprises an RNAse IIIA domain, an RNAse IIIb domain, and lacks a functional dsRBD. The second Dicer polypeptide lacks a DUF domain, a PAZ domain, and a DExD/H-box domain.

In some cases, a second Dicer polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1235 to about 1922, or amino acids 1296 to 1922, of the amino acid sequence set forth in FIG. **9**.

In some cases, e.g., where a second Dicer polypeptide lacks a functional dsRBD, the second Dicer polypeptide comprises an amino acid sequence having at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, amino acid sequence identity to amino acids 1235 to about 1772, or amino acids 1296 to 1772, of the amino acid sequence set forth in FIG. **9**. For example, in some embodiments, the second Dicer polypeptide lacks a dsRBD, e.g., lacks amino acids 1772-1912 of the amino acid sequence set forth in FIG. **9**, lacks amino acids 1772-1922 of the amino acid sequence set forth in FIG. **9**, or lacks a substantial portion of amino acids 1772-1912 such that the second Dicer polypeptide lacks a functional dsRBD.

The second Dicer polypeptide can have a length of from about 400 amino acids (aa) to about 950 aa, e.g., from about 400 aa to about 450 aa, from about 450 aa to about 500 aa, from about 500 aa to about 550 aa, from about 600 aa to about 650 aa, from about 650 aa to about 700 aa, from about 700 aa to about 750 aa, from about 750 aa to about 800 aa, from about 800 aa to about 850 aa, from about 850 aa to about 900 aa, or from about 900 aa to about 950 aa.

In some embodiments, the second Dicer polypeptide comprises one or more amino acid substitutions and/or deletions in the dsRBD, such that the dsRBD is non-functional.

In some embodiments, the second Dicer polypeptide is a chimeric Dicer polypeptide, e.g., the second Dicer polypeptide comprises a heterologous polypeptide. A heterologous polypeptide can be present at the carboxyl terminus, at the amino terminus, or at an internal site within the second Dicer polypeptide. Suitable heterologous polypeptides include, e.g., epitope tags, including, but not limited to, hemagglutinin, FLAG, and the like; proteins that provide for a detectable signal, including, but not limited to, fluorescent proteins, enzymes (e.g., β-galactosidase, alkaline phosphatase, luciferase, horse radish peroxidase, etc.), and the like; polypeptides that facilitate purification or isolation of the fusion protein, e.g., metal ion binding polypeptides such as 6 His tags, glutathione-S-transferase; etc.

Dicer Complex

The present disclosure provides a purified Dicer complex. A purified Dicer complex of the present disclosure is useful for producing small regulatory RNAs (e.g., siRNAs and miRNAs) from a dsRNA. A substrate dsRNA is contacted with a subject Dicer complex.

Compositions

The present invention provides a composition comprising a subject Dicer complex. A subject composition can comprise, in addition to the Dicer complex, one or more of: a salt, e.g., NaCl, $MgCl_2$, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a protease inhibitor; and the like.

In some embodiments, a Dicer complex present in a subject composition is pure, e.g., at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or more than 99% pure, where "% purity" means that the Dicer complex is the recited percent free from other proteins (e.g., proteins other than a subject Dicer complex), other macromolecules, or contaminants that may be present during the production of the Dicer complex.

Nucleic Acids

The present disclosure provides nucleic acids encoding the first and second Dicer polypeptides of a subject Dicer complex. A subject nucleic acid is recombinant. The present invention further provides a composition comprising a subject nucleic acid. In some cases, a subject nucleic acid comprises a nucleotide sequence encoding both the first and the second Dicer polypeptides of a subject Dicer complex. In other embodiments, two separate nucleic acids encode the two Dicer polypeptides; thus, the present disclosure provides a first nucleic acid comprising a nucleotide sequence encoding the first Dicer polypeptide of a subject Dicer complex; and a second nucleic acid comprising a nucleotide sequence encoding the second Dicer polypeptide of a subject Dicer complex.

In some embodiments, a subject nucleic acid is an expression construct, e.g., an expression vector comprising a nucleotide sequence encoding one or both of a first Dicer polypeptide and a second Dicer polypeptide of a subject Dicer complex, where the expression construct provides for production of the encoded modified Dicer polypeptide(s) in an appropriate host cell. Suitable expression vectors include, but are not limited to, baculovirus vectors, bacteriophage vectors, plasmids, phagemids, cosmids, fosmids, bacterial artificial chromosomes, viral vectors (e.g. viral vectors based on vaccinia virus, poliovirus, adenovirus, adeno-associated virus, SV40, herpes simplex virus, and the like), P1-based artificial chromosomes, yeast plasmids, yeast artificial chromosomes, and any other vectors specific for specific hosts of interest (such as *E. coli* and yeast).

Suitable vectors for the production of first and/or second Dicer polypeptides in a prokaryotic cell include plasmids of the types: pBR322-derived plasmids, pEMBL-derived plasmids, pEX-derived plasmids, pBTac-derived plasmids and pUC-derived plasmids for expression in prokaryotic cells, such as *Escherichia coli*. The following vectors are provided by way of example, for bacterial host cells: pQE vectors (Qiagen), pBluescript plasmids, pNH vectors, lambda-ZAP vectors (Stratagene); pTrc99a, pKK223-3, pDR540, and pRIT2T (Pharmacia). However, any other plasmid or other vector may be used so long as it is compatible with the host cell.

A number of vectors exist for the expression of recombinant proteins in yeast. For instance, YEP24, YIPS, YEP51, YEP52, pYES2, and YRP17 are cloning and expression vehicles useful in the introduction of genetic constructs into *Saccharomyces cerevisiae* (see, for example, Broach et al. (1983) in Experimental Manipulation of Gene Expression, ed. M. Inouye Academic Press, p. 83, incorporated by reference herein). These vectors can replicate in *E. coli* due the presence of the pBR322 ori, and in *S. cerevisiae* due to the replication determinant of the yeast 2 micron plasmid. In addition, drug resistance markers such as ampicillin can be used. In an illustrative embodiment, a one or both of the first and second Dicer polypeptides is produced recombinantly utilizing an expression vector generated by sub-cloning a nucleotide sequence encoding one or both of the first and second Dicer polypeptides of a subject Dicer complex.

In some embodiments, the expression construct comprises a mammalian expression vector. Suitable mammalian expression vectors include those that contain both prokaryotic sequences, to facilitate the propagation of the vector in bacteria, and one or more eukaryotic transcription units that are expressed in eukaryotic cells. The pcDNAI/amp, pcDNAI/neo, pRc/CMV, pSV2gpt, pSV2neo, pSV2-dhfr, pTk2, pRSVneo, pMSG, pSVT7, pko-neo and pHyg derived vectors are examples of mammalian expression vectors suitable for transfection of eukaryotic cells. Some of these vectors are modified with sequences from bacterial plasmids, such as pBR322, to facilitate replication and drug resistance selection in both prokaryotic and eukaryotic cells. Alternatively, derivatives of viruses such as the bovine papillomavirus (BPV-1), or Epstein-Ban virus (pHEBo, pREP-derived and p205) can be used for transient expression of proteins in eukaryotic cells. The various methods employed in the preparation of the plasmids and transformation of host organisms are well known in the art. For other suitable expression systems for both prokaryotic and eukaryotic cells, as well as general recombinant procedures, see Molecular Cloning: A Laboratory Manual, 2nd Ed., ed. by Sambrook, Fritsch and Maniatis (Cold Spring Harbor Laboratory Press: 1989) Chapters 16 and 17.

A first and/or a second Dicer polypeptide can be produced using an expression vector containing a nucleic acid encoding first and/or a second Dicer polypeptide, operably linked to at least one transcriptional regulatory sequence. Operably linked is intended to mean that the nucleotide sequence is linked to a regulatory sequence in a manner that allows expression of the nucleotide sequence. Regulatory sequences are art-recognized and are selected to direct expression of the encoded first and/or second Dicer protein. Accordingly, the term transcriptional regulatory sequence includes promoters, enhancers and other expression control elements. Such regulatory sequences are described in Goeddel; Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). For instance, any of a wide variety of expression control sequences, sequences that control the expression of a DNA sequence when operatively linked to it, may be used in these vectors to express DNA sequences encoding Dicer polypeptides to recombinantly produce a subject Dicer complex. Such useful expression control sequences, include, for example, a viral LTR, such as the LTR of the Moloney murine leukemia virus, the early and late promoters of SV40, adenovirus or cytomegalovirus immediate early promoter, the lac system, the trp system, the TAG or TRC system, T7 promoter whose expression is directed by T7 RNA polymerase, the major operator and promoter regions of phage X, polyhedron promoter, the control regions for fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase, e.g., Pho5, the promoters of the yeast a-mating factors, the polyhedron promoter of the baculovirus system and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof. It should be understood that the design of the expression vector may depend on such factors as the choice of the host cell to be transformed and/or the type of protein desired to be expressed.

Suitable promoters for use in prokaryotic host cells include, but are not limited to, a bacteriophage T7 RNA polymerase promoter; a trp promoter; a lac operon promoter; a hybrid promoter, e.g., a lac/tac hybrid promoter, a tac/trc hybrid promoter, a trp/lac promoter, a T7/lac promoter; a trc promoter; a tac promoter, and the like; an araBAD promoter; in vivo regulated promoters, such as an ssaG promoter or a related promoter (see, e.g., U.S. Patent Publication No. 20040131637), a pagC promoter (Pulkkinen and Miller, *J. Bacteriol.,* 1991: 173(1): 86-93; Alpuche-Aranda et al., PNAS, 1992; 89(21): 10079-83), a nirB promoter (Harborne et al. (1992) *Mol. Micro.* 6:2805-2813), and the like (see, e.g., Dunstan et al. (1999) *Infect. Immun.* 67:5133-5141; McKelvie et al. (2004) *Vaccine* 22:3243-3255; and Chatfield et al. (1992) *Biotechnol.* 10:888-892); a sigma70 promoter, e.g., a consensus sigma70 promoter (see, e.g., GenBank Accession Nos. AX798980, AX798961, and AX798183); a stationary phase promoter, e.g., a dps promoter, an spy promoter, and the like; a promoter derived from the pathogenicity island SPI-2 (see, e.g., WO96/17951); an actA promoter (see, e.g., Shetron-Rama et al. (2002) *Infect. Immun.* 70:1087-1096); an rpsM promoter (see, e.g., Valdivia and Falkow (1996). *Mol. Microbiol.* 22:367); a tet promoter (see, e.g., Hillen, W. and Wissmann, A. (1989) In Saenger, W. and Heinemann, U. (eds), *Topics in Molecular and Structural Biology, Protein—Nucleic Acid Interaction*. Macmillan, London, UK, Vol. 10, pp. 143-162); an SP6 promoter (see, e.g., Melton et al. (1984) *Nucl. Acids Res.* 12:7035-7056); and the like.

Non-limiting examples of suitable eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Suitable promoters for expression in yeast include, but are not limited to, CYC1, HIS3, GAL1, GAL10, ADH1, PGK, PHO5, GAPDH, ADC1, TRP1, URA3, LEU2, ENO, and TP1; and, e.g., AOX1 (e.g., for use in *Pichia*).

In some embodiments, the promoter is an inducible promoter. Suitable inducible promoters include, but are not limited to, the pL of bacteriophage λ; Plac; Ptrp; Ptac (Ptrp-lac hybrid promoter); an isopropyl-beta-D-thiogalactopyranoside (IPTG)-inducible promoter, e.g., a lacZ promoter; a tetracycline-inducible promoter; an arabinose inducible promoter, e.g., P_{BAD} (see, e.g., Guzman et al. (1995) *J. Bacteriol.* 177:4121-4130); a xylose-inducible promoter, e.g., Pxyl (see, e.g., Kim et al. (1996) *Gene* 181:71-76); a GAL1 promoter; a tryptophan promoter; a lac promoter; an alcohol-inducible promoter, e.g., a methanol-inducible promoter, an ethanol-inducible promoter; a raffinose-inducible promoter; a heat-inducible promoter, e.g., heat inducible lambda P_L promoter, a promoter controlled by a heat-sensitive repressor (e.g., CI857-repressed lambda-based expression vectors; see, e.g., Hoffmann et al. (1999) *FEMS Microbiol Lett.* 177(2):327-34); and the like.

In yeast, a number of vectors containing constitutive or inducible promoters may be used. For a review see, Current Protocols in Molecular Biology, Vol. 2, 1988, Ed. Ausubel, et al., Greene Publish. Assoc. & Wiley Interscience, Ch. 13; Grant, et al., 1987, Expression and Secretion Vectors for Yeast, in Methods in Enzymology, Eds. Wu & Grossman, 31987, Acad. Press, N.Y., Vol. 153, pp. 516-544; Glover, 1986, DNA Cloning, Vol. II, IRL Press, Wash., D.C., Ch. 3; and Bitter, 1987, Heterologous Gene Expression in Yeast, Methods in Enzymology, Eds. Berger & Kimmel, Acad. Press, N.Y., Vol. 152, pp. 673-684; and The Molecular Biology of the Yeast *Saccharomyces,* 1982, Eds. Strathern et al., Cold Spring Harbor Press, Vols. I and II. A constitutive yeast promoter such as ADH or LEU2 or an inducible promoter such as GAL may be used (Cloning in Yeast, Ch. 3, R. Rothstein In: DNA Cloning Vol. 11, A Practical Approach, Ed. D M Glover, 1986, IRL Press, Wash., D.C.). Alternatively, vectors may be used which promote integration of foreign DNA sequences into the yeast chromosome.

Compositions

The present invention provides a composition comprising a subject nucleic acid(s). A subject composition can comprise, in addition to a subject nucleic acid(s), one or more of: a salt, e.g., NaCl, $MgCl_2$, KCl, $MgSO_4$, etc.; a buffering agent, e.g., a Tris buffer, N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid) (HEPES), 2-(N-Morpholino)ethanesulfonic acid (MES), 2-(N-Morpholino)ethanesulfonic acid sodium salt (MES), 3-(N-Morpholino)propanesulfonic acid (MOPS), N-tris[Hydroxymethyl]methyl-3-aminopropanesulfonic acid (TAPS), etc.; a solubilizing agent; a detergent, e.g., a non-ionic detergent such as Tween-20, etc.; a nuclease inhibitor; glycerol; and the like.

Genetically Modified Host Cells

The present invention provides genetically modified host cells comprising a subject nucleic acid(s). Suitable host cells include, e.g., prokaryotic host cells (e.g., prokaryotic cells in vitro). The present invention further provides composition comprising a subject genetically modified host cell.

Suitable prokaryotic cells include, but are not limited to, any of a variety of laboratory strains of *Escherichia coli, Lactobacillus* sp., *Salmonella* sp., *Shigella* sp., and the like. See, e.g., Carrier et al. (1992) *J. Immunol.* 148:1176-1181; U.S. Pat. No. 6,447,784; and Sizemore et al. (1995) *Science* 270:299-302. Examples of *Salmonella* strains which can be employed in the present invention include, but are not limited to, *Salmonella typhi* and *S. typhimurium*. Suitable *Shigella* strains include, but are not limited to, *Shigella flexneri, Shigella sonnei*, and *Shigella disenteriae*. Typically, the laboratory strain is one that is non-pathogenic. Non-limiting examples of other suitable bacteria include, but are not limited to, *Bacillus subtilis, Pseudomonas pudita, Pseudomonas aeruginosa, Pseudomonas mevalonii, Rhodobacter sphaeroides, Rhodobacter capsulatus, Rhodospirillum rubrum, Rhodococcus* sp., and the like. In some embodiments, the host cell is *Escherichia coli.*

Suitable methods of genetic modification of a host cell include viral infection, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate precipitation, direct microinjection, and the like. The choice of method is generally dependent on the type of cell being transformed and the circumstances under which the transformation is taking place (i.e. in vitro, ex vivo, or in vivo). A general discussion of these methods can be found in Ausubel, et al, Short Protocols in Molecular Biology, 3rd ed., Wiley & Sons, 1995. To generate a subject genetically modified host cell, a subject nucleic acid is introduced stably or transiently into a host cell, using established techniques, including, but not limited to, electroporation, lithium acetate transformation, calcium phosphate precipitation, DEAE-dextran mediated transfection, liposome-mediated transfection, and the like. For stable transformation, a nucleic acid will generally further include a selectable marker, e.g., any of several well-known selectable markers such as neomycin resistance, ampicillin resistance, tetracycline resistance, chloramphenicol resistance, kanamycin resistance, and the like.

Compositions

The present invention provides a composition comprising a subject genetically modified host cell. A subject composition comprises a subject genetically modified host cell, and will in some embodiments comprise one or more further components, which components are selected based in part on the intended use of the genetically modified host cell, storage considerations, etc. Suitable components include, but are not limited to, salts; buffers; stabilizers; protease-inhibiting agents; nuclease-inhibiting agents; cell membrane- and/or cell wall-preserving compounds, e.g., glycerol, dimethylsulfoxide, etc.; nutritional media appropriate to the cell; and the like. In some embodiments, the cells are lyophilized.

Production of a Subject Dicer Complex

A host cell is genetically modified with a subject nucleic acid, such that one or both of the first and second polypeptides of a subject Dicer complex is produced in the genetically modified host cell, and the encoded first and/or second Dicer polypeptide is(are) produced by the cell. The genetically modified host cell is cultured in vitro under suitable conditions such that one or both of the first and second polypeptides of a subject Dicer complex is produced. Where the nucleotide sequence encoding one or both of the first and second polypeptides of a subject Dicer complex is operably linked to an inducible promoter, an inducer is added to the culture medium in which the genetically modified host cell is cultured.

The first and/or the second Dicer polypeptides can be recovered and isolated from the genetically modified host cell; and allowed to form a complex outside the cell. In some embodiments, one or both of the first and second polypeptides of a subject Dicer complex polypeptide is purified, e.g., is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% pure. Any convenient protein purification procedures may be employed, where suitable protein purification methodologies are described in Guide to Protein Purification, (Deuthser ed.) (Academic Press, 1990). For example, a lysate may be prepared from a genetically modified host cell that expresses one or both of the first and second polypeptides of a subject Dicer complex, and purified using any of a number of standard protein purification methods, e.g., high performance liquid chromatography, size exclusion chromatography, gel electrophoresis, affinity chromatography, and the like.

Utility

A subject Dicer complex is useful for producing small regulatory RNAs, which in turn are useful in a number of applications, including basic research applications, drug screening/target validation, large scale functional library screening, and therapeutic applications. Thus, the present disclosure provides methods of producing a small regulatory RNA molecule from a substrate dsRNA molecule. Small regulatory RNA molecules that can be produced using a subject method include siRNA and miRNA.

Methods of Producing a Small Regulatory RNA Molecule

The present invention provides methods of producing small regulatory RNA from a substrate dsRNA molecule, the methods generally involving contacting the substrate dsRNA molecule with a subject Dicer complex, where the Dicer complex efficiently produces a small regulatory RNA using the substrate dsRNA molecule. The methods described below are directed to producing siRNA; however, a subject method can be adapted for producing miRNA.

In some embodiments, a subject method provides for production of a plurality of small regulatory RNA molecules, e.g., a plurality of siRNA molecules or a plurality of miRNA molecules. By "plurality" is meant at least 2, at least about 5, or at least about 10, where the number of distinct siRNA or miRNA molecules produced from a given substrate dsRNA molecule in the subject methods can depend on the length of the substrate dsRNA molecule, but may be as high as about 25 or higher, e.g., about 100, or about 400 or higher.

The siRNA or miRNA product molecules can range in length from about 10 nucleotides (nt) to about 25 nt, e.g., from about 10 nt to about 15 nt, from about 15 nt to about 20 nt, or from about 20 nt to about 25 nt. In some embodiments, a subject Dicer complex produces siRNA product molecules having a length of from about 19 nt to about 24 nt, from about 20 nt to about 24 nt, from about 21 nt to about 24 nt, or from about 21 nt to about 23 nt. In some embodiments, a subject Dicer complex produces siRNA product molecules, where at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, of the siRNA molecules have a length of from 21 nt to 23 nt.

A subject Dicer complex is contacted with a substrate dsRNA molecule. The length of the parent dsRNA molecule can vary, but generally the length is at least about 300 bp, at least about 500 bp, or at least about 1000 bp, where the length may be as long as about 2000 bp or longer, but often does not exceed about 8000 bp, e.g., about 6000 bp.

The dsRNA substrate can comprise two hybridized strands of polymerized ribonucleotide. The dsRNA substrate can include modifications to either the phosphate-sugar backbone or the nucleoside. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or a sulfur heteroatom. Modifications in RNA structure may be tailored to allow specific genetic inhibition while avoiding an adverse response in the cell harboring the RNA. Likewise, bases may be modified to block the activity of adenosine deaminase. The dsRNA substrate may be produced enzymatically or by partial/total organic synthesis, any modified ribonucleotide can be introduced by in vitro enzymatic or organic synthesis.

The dsRNA substrate is formed by a single self-complementary RNA strand or by two complementary RNA strands. dsRNA substrates comprising a nucleotide sequence identical to a portion of a target gene may be employed. RNA sequences with insertions, deletions, and single point mutations relative to the target sequence are also of interest. Thus, sequence identity may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). In some embodiments, there is at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, at least about 99%, or 100%, sequence identity between the siRNA or miRNA and the portion of a target gene may be of interest. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. hybridization for 12-16 hours; followed by washing; or conditions that are at least as stringent as these representative conditions). The length of the identical nucleotide sequences may be, for example, at least about 25, about 50, about 100, about 200, about 300 or about 400 bases or longer. In certain embodiments, the dsRNA substrate is from about 400 to about 800 bases in length. In certain embodiments 100% sequence identity between the RNA and the target gene is not required to practice inhibition applications of the invention. Thus the invention has the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence.

The dsRNA substrate can be synthesized either in vivo or in vitro. Furthermore, the dsRNA substrate can be synthesized in vitro in a living cell, or in a cell-free in vitro system. Endogenous polymerase of the cell can mediate transcription in vivo, or cloned RNA polymerase can be used for transcription in vivo or in vitro. For transcription from a transgene in vivo or an expression construct, a regulatory region (e.g., promoter, enhancer, silencer, splice donor and acceptor, polyadenylation) may be used to transcribe the dsRNA strand (or strands). In some embodiments, the RNA strands of the dsRNA substrate are polyadenylated. In other embodiments, the RNA strands of the dsRNA substrate are not polyadenylated. In some embodiments, the RNA strands of the dsRNA substrate are capable of being translated into a polypeptide by a cell's translational apparatus or in a cell-free in vitro translation system. In some embodiments, the RNA strands of the dsRNA substrate are not capable of being translated into a polypeptide by a cell's translational apparatus or in a cell-free in vitro translation system.

The dsRNA substrate can be chemically or enzymatically synthesized by manual or automated reactions. The dsRNA substrate can be synthesized by a cellular RNA polymerase or a bacteriophage RNA polymerase (e.g., T3, T7, or SP6), e.g., using an expression construct encoding the dsRNA as template. The use and production of expression constructs are known in the art (see WO 97/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5,712,135, 5,789,214, and 5,804,693; and the references cited therein). If synthesized chemically or by in vitro enzymatic synthesis, the RNA can be purified prior to introduction into the cell. For example, RNA can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography or a combination thereof. Alternatively, the dsRNA construct may be used with no or a minimum of purification to avoid losses due to sample processing. The dsRNA construct may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

In some embodiments, at least about 60%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99%, of the substrate dsRNA is cleaved to produce an miRNA or siRNA product.

In the reaction composition (e.g., the composition comprising a subject Dicer complex and a dsRNA substrate), the amount of Dicer complex present in the composition can vary, and can be in a range of from about 20 ng/μl to about 160 ng/μl, e.g., from about 20 ng/μl to about 40 ng/μl, from about 40 ng/μl to about 60 ng/μl, from about 60 ng/μl to about 80 ng/μl, from about 80 ng/μl to about 100 ng/μl, from about 100 ng/μl to about 120 ng/μl, from about 120 ng/μl to about 140 ng/μl, or from about 140 ng/μl to about 160 ng/μl.

In some embodiments, the reaction composition (e.g., the composition comprising a subject Dicer complex and a dsRNA substrate) is an aqueous composition, where the composition may include one or more additional components, e.g., buffers; salts such as NaCl, $MgCl_2$, and the like; EDTA; DTT; ATP; and the like.

As discussed above, a subject method comprises contacting a subject Dicer complex with a substrate dsRNA in a reaction composition that is then maintained under conditions sufficient to produce the desired siRNA or miRNA product. In some embodiments, a subject method is a cell-free in vitro method, by which is meant that the method occurs in a cell free environment, e.g., not inside of a cell or in the presence of cells. As such, in some embodiments, a subject method involves producing a product composition comprising an siRNA product or a miRNA product, where the product composition is produced by contacting a substrate dsRNA and a subject Dicer complex, as described above, where the product composition is produced in a cell-free in vitro reaction, i.e., in vitro and outside of a cell.

In some embodiments, a subject Dicer complex and a substrate dsRNA are contacted in reaction composition that includes a sufficient amount of Mg^{2+} to ensure adequate Dicer activity, where the amount of Mg^{2+} can range from about 0.5 mM to about 1.0 mM, or from about 2.5 mM to about 5.0 mM. In some embodiments, the reaction composition is free of ATP, and in other embodiments, 1 mM ATP is used in the reaction composition.

The reaction mixture is typically maintained under incubation conditions sufficient to produce the desired small regulatory RNA product. The reaction mixture is typically maintained at a temperature that ranges from about 30° C. to about 37° C., e.g., from about 35° C. to about 37° C. The reaction is carried out for a period of time ranging from about 15 minutes to about 24 hours, e.g., from about 15 minutes to about 30 minutes, from about 30 minutes to about 60 minutes, from about 1 hour to about 2 hours, from about 2 hours to about 4 hours, from about 4 hours to about 8 hours, from about 8 hours to about 12 hours, from about 12 hours to about 16 hours, or from about 16 hours to about 24 hours.

The small regulatory RNA product, e.g., the siRNA product or the miRNA produce, produced by a subject method may be used as is or further processed prior to use, e.g., separated from other components of the reaction mixture, e.g., the Dicer comlex, any remaining dsRNA substrate, salts, buffers, etc. Any convenient separation protocol may be employed, including gel purification, chromatographic separation based on molecular weight or affinity resins, and classical precipitation, and the like.

Research Applications

A small regulatory RNA can be used for modifying biological functions in a cell (e.g., a cell growing as a single-cell suspension in vitro; a cell in a multicellular organism; etc.), such as for example, RNA interference, gene knockdown or knockout, generating expression mutants, modulating cell growth, differentiation, signaling or a combination thereof. Thus, in some embodiments, a subject method involves: a) producing an siRNA using a subject method (i.e., using a subject Dicer complex); and b) introducing the siRNA so produced into a cell (e.g., into a cell in vitro; or into a non-human cell in a multi-cellular organism in vivo).

One representative utility is a method of identifying gene function in an organism, e.g., higher eukaryotes comprising the use of the product siRNA to inhibit the activity of a target gene of previously unknown function. Instead of the time consuming and laborious isolation of mutants by traditional genetic screening, functional genomics using the subject product siRNA determines the function of uncharacterized genes by employing the siRNA to reduce the amount and/or alter the timing of target gene activity. The product siRNA can be used in determining potential targets for pharmaceutics, understanding normal and pathological events associated with development, determining signaling pathways responsible for postnatal development/aging, and the like. The increasing speed of acquiring nucleotide sequence information from genomic and expressed gene sources, including total sequences for mammalian genomes, can be coupled with use of the product siRNA to determine gene function in a cell or in a whole organism. The preference of different organisms to use particular codons, searching sequence databases for related gene products, correlating the linkage map of genetic traits with the physical map from which the nucleotide sequences are derived, and artificial intelligence methods may be used to define putative open reading frames from the nucleotide sequences acquired in such sequencing projects.

A simple representative assay involves inhibition of gene expression according to the partial sequence available from an expressed sequence tag (EST). Functional alterations in growth, development, metabolism, disease resistance, or other biological processes would be indicative of the normal role of the EST's gene product.

The ease with which the product siRNA construct can be introduced into an intact cell/organism containing the target gene allows the siRNA products to be used in high throughput screening (HTS). For example, individual clones from the library can be replicated and then isolated in separate reactions, but preferably the library is maintained in individual reaction vessels (e.g., a 96-well microtiter plate) to minimize the number of steps required to practice the invention and to allow automation of the process. Solutions containing the product siRNAs that are capable of inhibiting the different expressed genes can be placed into individual wells positioned on a microtiter plate as an ordered array, and intact cells/organisms in each well can be assayed for any changes or modifications in behavior or development due to inhibition of target gene activity.

The siRNA can be fed directly to, injected into, the cell/organism containing the target gene. The siRNA may be directly introduced into the cell (i.e., intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, or may be introduced by bathing an organism in a solution containing the siRNA. Methods for oral introduction include direct mixing of RNA with food of the organism. Physical methods of introducing nucleic, acids include injection directly into the cell or extracellular injection into the organism of an RNA solution. The siRNA may be introduced in an amount that allows delivery of at least one copy per cell. Higher doses (e.g., at least 5, 10, 100, 500 or 1000 copies per cell) of siRNA material may yield more effective inhibition; lower doses may also be useful for specific applications. Inhibition is sequence-specific in that nucleotide sequences corresponding to the duplex region of the RNA are targeted for genetic inhibition.

The function of the target gene can be assayed from the effects it has on the cell/organism when gene activity is inhibited. This screening could be amenable to small subjects that can be processed in large number, for example, tissue culture cells derived from invertebrates or vertebrates (e.g., mammals, such as murines, non-human primates, and humans).

If a characteristic of an organism is determined to be genetically linked to a polymorphism through RFLP or QTL analysis, the present invention can be used to gain insight regarding whether that genetic polymorphism might be directly responsible for the characteristic. For example, a fragment defining the genetic polymorphism or sequences in the vicinity of such a genetic polymorphism can be amplified to produce a dsRNA from which siRNA is prepared according to the subject methods, which siRNA can be introduced to the organism or cell, and whether an alteration in the characteristic is correlated with inhibition can be determined.

A Dicer complex of the present disclosure is useful in allowing the inhibition of essential genes. Such genes may be required for cell or organism viability at only particular stages of development or cellular compartments. The functional equivalent of conditional mutations may be produced by inhibiting activity of the target gene when or where it is not required for viability. The invention allows addition of siRNA at specific times of development and locations in the organism without introducing permanent mutations into the target genome.

In situations where alternative splicing produces a family of transcripts that are distinguished by usage of characteristic exons, an siRNA can target inhibition through the appropriate exons to specifically inhibit or to distinguish among the functions of family members.

Therapeutic Applications

An siRNA produced using a subject method also finds use in a variety of therapeutic applications in which it is desired to selectively modulate one or more target genes in a host, e.g., a whole animal, or a portion thereof, e.g., a tissue, an organ, etc, as well as in cells present such an animal, tissue, or organ. In such methods, an effective amount of an siRNA is administered to the host or target portion thereof. By "effective amount" is meant a dosage sufficient to selectively modulate expression of the target gene(s), as desired. As indicated above, in many embodiments of this type of application, methods are employed to reduce/inhibit expression of one or more target genes in the host or portion thereof in order to achieve a desired therapeutic outcome.

In some embodiments, a subject method comprises: preparing an siRNA according to a subject method (i.e., using a subject Dicer complex); and administering an effective amount of the siRNA to an individual in need thereof.

Depending on the nature of the condition being treated, the target gene may be a gene derived from the cell, an endogenous gene, a pathologically mutated gene, e.g. a cancer-causing gene, one or more genes whose expression causes or is related to heart disease, lung disease, Alzheimer's disease, Parkinson's disease, diabetes, arthritis, etc.; a transgene, or a gene of a pathogen which is present in the cell after infection thereof, e.g., a viral (e.g., HIV-Human Immunodeficiency Virus; Hepatitis B virus; Hepatitis C virus; Herpes-simplex virus-1 and -2; Varicella Zoster (Chicken pox and Shingles); Rhinovirus (common cold and flu); any other viral form); or bacterial pathogen. Depending on the particular target gene and the dose of siRNA delivered, the procedure may provide partial or complete loss of function for the target gene. Lower doses of injected material and longer times after administration of siRNA may result in inhibition in a smaller fraction of cells.

An siRNA produced using a subject method finds use in the treatment of a variety of conditions in which the modulation of target gene expression in a mammalian host is desired. By treatment is meant that at least an amelioration of the symptoms associated with the condition afflicting the host is achieved, where amelioration is used in a broad sense to refer to at least a reduction in the magnitude of a parameter, e.g. symptom, associated with the condition being treated. As such, treatment also includes situations where the pathological condition, or at least symptoms associated therewith, are completely inhibited, e.g. prevented from happening, or stopped, e.g. terminated, such that the host no longer suffers from the condition, or at least the symptoms that characterize the condition.

A variety of hosts are treatable using an siRNA. Generally such hosts are "mammals" or "mammalian," where these terms are used broadly to describe organisms which are within the class mammalia, including the orders carnivore (e.g., dogs and cats), rodentia (e.g., mice, guinea pigs, and rats), and primates (e.g., humans, and non-human primates such as chimpanzees and monkeys). In some embodiments, the hosts will be humans.

The present disclosure is not limited to modulation of expression of any specific type of target gene or nucleotide sequence. Representative classes of target genes of interest include but are not limited to: developmental genes (e.g., adhesion molecules, cyclin kinase inhibitors, cytokines/lymphokines and their receptors, growth/differentiation factors and their receptors, neurotransmitters and their receptors); oncogenes (e.g., ABLI, BCLI, BCL2, BCL6, CBFA2, CBL, CSFIR, ERBA, ERBB, EBRB2, ETSI, ETS1, ETV6, FOR, FOS, FYN, HCR, HRAS, JUN, KRAS, LCK, LYN, MDM2, MLL, MYB, MYC, MYCLI, MYCN, NRAS, PIM 1, PML, RET, SRC, TALI, TCL3, and YES); tumor suppressor genes (e.g., APC, BRCA1, BRCA2, MADH4, MCC, NF1, NF2, RB 1, TP53, and WTI); and enzymes (e.g., ACC synthases and oxidases, ACP desaturases and hydroxylases, ADP-glucose pyrophorylases, ATPases, alcohol dehydrogenases, amylases, amyloglucosidases, catalases, cellulases, chalcone synthases, chitinases, cyclooxygenases, decarboxylases, dextrinases, DNA and RNA polymerases, galactosidases, glucanases, glucose oxidases, granule-bound starch synthases, GTPases, helicases, hemicellulases, integrases, inulinases, invertases, isomerases, kinases, lactases, Upases, lipoxygenases, lysozymes, nopaline synthases, octopine synthases, pectinesterases, peroxidases, phosphatases, phospholipases, phosphorylases, phytases, plant growth regulator synthases, polygalacturonases, proteinases and peptidases, pullanases, recombinases, reverse transcriptases, RUBISCOs, topoisomerases, and xylanases);

chemokines (e.g. CXCR4, CCR5); the RNA component of telomerase; vascular endothelial growth factor (VEGF); VEGF receptor; tumor necrosis factors nuclear factor kappa B; transcription factors; cell adhesion molecules; Insulin-like growth factor; transforming growth factor beta family members; cell surface receptors; RNA binding proteins (e.g. small nucleolar RNAs, RNA transport factors); translation factors; telomerase reverse transcriptase); etc.

The siRNA can be introduced into the target cell(s) using any convenient protocol, where the protocol will vary depending on whether the target cells are in vitro or in vivo.

Where the target cells are in vivo, the siRNA can be administered to the host comprising the cells using any convenient protocol, where the protocol employed is typically a nucleic acid administration protocol, where a number of different such protocols are known in the art. The following discussion provides a review of representative nucleic acid administration protocols that may be employed. The nucleic acids may be introduced into tissues or host cells by any number of routes, including microinjection, or fusion of vesicles. Jet injection may also be used for intra-muscular administration, as described by Furth et al. (1992), *Anal Biochem* 205:365-368. The nucleic acids may be coated onto gold microparticles, and delivered intradermally by a particle bombardment device, or "gene gun" as described in the literature (see, for example, Tang et al. (1992), *Nature* 356:152-154), where gold microprojectiles are coated with the DNA, then bombarded into skin cells.

For example, the d-siRNA agent can be fed directly to, injected into, the host organism containing the target gene. The agent may be directly introduced into the cell (i.e., intracellularly); or introduced extracellularly into a cavity, interstitial space, into the circulation of an organism, introduced orally, etc. Methods for oral introduction include direct mixing of RNA with food of the organism. Physical methods of introducing nucleic acids include injection directly into the cell or extracellular injection into the organism of an RNA solution.

In certain embodiments, a hydrodynamic nucleic acid administration protocol is employed. Where the agent is a ribonucleic acid, the hydrodynamic ribonucleic acid administration protocol described in detail below is of particular interest. Where the agent is a deoxyribonucleic acid, the hydrodynamic deoxyribonucleic acid administration protocols described in Chang et al., J. Virol. (2001) 75:3469-3473; Liu et al., Gene Ther. (1999) 6:1258-1266; Wolff et al., Science (1990) 247: 1465-1468; Zhang et al., Hum. Gene Ther. (1999) 10:1735-1737: and Zhang et al., Gene Ther. (1999) 7:1344-1349; are of interest.

Additional nucleic acid delivery protocols of interest include, but are not limited to: those described in U.S. Pat. Nos. 5,985,847 and 5,922,687 (the disclosures of which are herein incorporated by reference); Acsadi et al., New Biol. (1991) 3:71-81; Hickman et al., Hum. Gen. Ther. (1994) 5:1477-1483; and Wolff et al., Science (1990) 247: 1465-1468; etc.

An siRNA (also referred to as an "agent" or an "active agent") can be administered to the host using any convenient means capable of resulting in the desired modulation of target gene expression. Thus, the agent can be incorporated into a variety of formulations for therapeutic administration. More particularly, the agents can be formulated into pharmaceutical compositions by combination with appropriate, pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants and aerosols. As such, administration of the agents can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intracheal, etc., administration.

In pharmaceutical dosage forms, the agents may be administered alone or in appropriate association, as well as in combination, with other pharmaceutically active compounds. The following methods and excipients are merely exemplary and are in no way limiting.

Suitable delivery reagents for administration of an siRNA include the Minis Transit TKO lipophilic reagent; lipofectin; lipofectamine; cellfectin; polycations (e.g., polylysine); and liposomes.

For oral preparations, the agents can be used alone or in combination with appropriate additives to make tablets, powders, granules or capsules, for example, with conventional additives, such as lactose, mannitol, corn starch or potato starch; with binders, such as crystalline cellulose, cellulose derivatives, acacia, corn starch or gelatins; with disintegrators, such as corn starch, potato starch or sodium carboxymethylcellulose; with lubricants, such as talc or magnesium stearate; and if desired, with diluents, buffering agents, moistening agents, preservatives and flavoring agents.

The agents can be formulated into preparations for injection by dissolving, suspending or emulsifying them in an aqueous or nonaqueous solvent, such as vegetable or other similar oils, synthetic aliphatic acid glycerides, esters of higher aliphatic acids or propylene glycol; and if desired, with conventional additives such as solubilizers, isotonic agents, suspending agents, emulsifying agents, stabilizers and preservatives.

The agents can be utilized in aerosol formulation to be administered via inhalation. The compounds of the present invention can be formulated into pressurized acceptable propellants such as dichlorodifluoromethane, propane, nitrogen and the like.

Furthermore, the agents can be made into suppositories by mixing with a variety of bases such as emulsifying bases or water-soluble bases. An active agent can be administered rectally via a suppository. The suppository can include vehicles such as cocoa butter, carbowaxes and polyethylene glycols, which melt at body temperature, yet are solidified at room temperature.

Unit dosage forms for oral or rectal administration such as syrups, elixirs, and suspensions may be provided wherein each dosage unit, for example, teaspoonful, tablespoonful, tablet or suppository, contains a predetermined amount of the composition containing one or more agents. Similarly, unit dosage forms for injection or intravenous administration may comprise the agent(s) in a composition as a solution in sterile water, normal saline or another pharmaceutically acceptable carrier.

The term "unit dosage form," as used herein, refers to physically discrete units suitable as unitary dosages for human and non-human animal subjects, each unit containing a predetermined quantity of an active agent calculated in an amount sufficient to produce the desired effect in association with a pharmaceutically acceptable diluent, carrier or vehicle.

The pharmaceutically acceptable excipients, such as vehicles, adjuvants, carriers or diluents, are readily available to the public. Moreover, pharmaceutically acceptable auxiliary substances, such as pH adjusting and buffering agents, tonicity adjusting agents, stabilizers, wetting agents and the like, are readily available to the public.

Those of skill in the art will readily appreciate that dose levels can vary as a function of the specific compound, the nature of the delivery vehicle, and the like. Preferred dosages for a given active agent are readily determinable by those of skill in the art by a variety of means.

Kits

The present disclosure provides a kit for producing a subject Dicer complex. A subject kit comprises: a) a first recombinant expression vector comprising a nucleotide sequence encoding a first Dicer polypeptide, wherein the first Dicer polypeptide comprises a DUF and a PAZ domain; and b) a second recombinant expression vector comprising a nucleotide sequence encoding a second Dicer polypeptide comprises an RNAse IIIA domain, an RNase IIIb domain, and a double-stranded RNA binding domain. The first and the second Dicer polypeptides are amply described above. The components can be in separate containers.

In addition to above-mentioned components, a subject kit can include instructions for using the components of the kit to practice a subject method for producing a Dicer complex. The instructions for practicing a subject method are generally recorded on a suitable recording medium. For example, the instructions may be printed on a substrate, such as paper or plastic, etc. As such, the instructions may be present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In other embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. compact disc-read only memory (CD-ROM), digital versatile disk (DVD), diskette, etc. In yet other embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source, e.g. via the internet, are provided. An example of this embodiment is a kit that includes a web address where the instructions can be viewed and/or from which the instructions can be downloaded. As with the instructions, this means for obtaining the instructions is recorded on a suitable substrate.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.) but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Celsius, and pressure is at or near atmospheric. Standard abbreviations may be used, e.g., bp, base pair(s); kb, kilobase(s); pl, picoliter(s); s or sec, second(s); min, minute(s); h or hr, hour(s); aa, amino acid(s); kb, kilobase(s); bp, base pair(s); nt, nucleotide(s); i.m., intramuscular(ly); i.p., intraperitoneal (ly); s.c., subcutaneous(ly); and the like.

Example 1

Dicer Complex

Experimental Procedures

RNA Substrates

All of the RNA oligonucleotides ("oligos") with exception of pre-hlet-7a-1 listed below were synthesized by IDT (Integrated DNA Technologies, Inc, Coralville, Iowa). All RNA oligos were purified by 16% urea-polyacrylamide gel electrophoresis (PAGE) before use. Human pre-let-7a-1 hairpin RNA (pre-hlet-7a-1, 73 nt) was transcribed in vitro by T7 RNA polymerase from a construct containing a double ribozyme system to ensure homogeneous 5' and 3' ends (29). An artificial hairpin RNA (37ab-loop, 79 nt) was made by the ligation of 37a-loop and 5'-phosphated 37b-loop (see below) with T4 RNA ligase from BioLabs (New England BioLabs, Inc, Beverly, Mass.). The 37ab-loop RNA was constructed such that it contains a perfectly matched stem from 37ab (see below) and a terminal loop from pre-hlet-7a-1. The RNA oligos of 37a and 37b can form a perfectly matched duplex (37ab). The RNA oligos of 21a and 21b were annealed to form duplex siRNA. The oligos of hlet7-stem-a and hlet7-stem-b can form a stem (hlet7-stem) from pre-hlet7 after annealing. For both filter binding and dicing assays, the purified RNA substrates were 5'-end labeled with ^{32}P using T4 polynucleotide kinase (New England Biolabs, Inc. Beverly, Mass.), gel-purified and annealed before use. The sequences of all of RNA substrates used in this study are:

```
pre-hlet-7a-1:
                                           (SEQ ID NO: 9)
5'-UGAGGUAGUAGGUUGUAUAGUUUUAGGGUCACACCCACCACUGGGAG

AUAACUAUACAAUCUACUGUCUUACC-3';

hlet7-stem-a:
                                          (SEQ ID NO: 10)
5'-UGAGGUAGUAGGUUGUAUAGUUUGAAAGUUCACGAUU-3';

hlet7-stem-b:
                                          (SEQ ID NO: 11)
5'-AAUCGUGAACUUUCAAACUAUACAAUCUACUGUCUUACC-3';

37a-loop:
                                          (SEQ ID NO: 14)
5'-UGAGGUAGUAGGUUGUAUAGUUUGAUUAGGGUCACACCCACC-3';

37b-loop:
                                          (SEQ ID NO: 15)
5'-P-ACUGGGAGAUUCAAACUAUACAACCUACUACCUCAUU-3';

37a:
                                          (SEQ ID NO: 10)
5'-UGAGGUAGUAGGUUGUAUAGUUUGAAAGUUCACGAUU-3';

37b:
                                          (SEQ ID NO: 12)
5'-UCGUGAACUUUCAAACUAUACAACCUACUACCUCAUU-3';

pre-miR20a:
                                          (SEQ ID NO: 16)
5'UAAAGUGCUUAUAGUGCAGGUAGUGUGUAGCCAUCUACUGCAUUACGA

GCACUUAAAG-3';

21a:
                                          (SEQ ID NO: 17)
5'-UAUACAAUGUGCUAGCUUUCU-3';
and

21b:
                                          (SEQ ID NO: 18)
5'-AAAGCUAGCACAUUGUAUAGU-3'.
```

Dicer Constructs for Sf9 and Bacterial Expression

To structurally probe hDcr and obtain its globular fragments, limited proteolysis was performed with endoproteinase Glu-C(Sigma-Aldrich, St. Louis, Mo.). Specifically, 60 ng of Glu-C was incubated with 30 μg of hDcr on ice for 60 min. The proteolytic fragments were separated on a 10% sodium dodecyl sulfate-PAGE (SDS-PAGE) and were then either stained with Coomassie Brilliant Blue and cut for MassSpec or transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, Mass.) for Edman degradation sequencing.

The N-terminal (hDcr-N: 1-1068) and C-terminal (hDcr-C: 1235-1922) fragments were co-expressed in SF9 cells transfected with their baculoviruses as described previously (7). The bacteria-expression constructs were designed based on the alignment data of published Dicer sequence (4, 10) (ATPase/helicase (ATPase/Hel): 1-604; DUF283-PAZ (DP): 605-1068; hDcr-C: 1235-1922; and hDcr-CΔRBD: 1235-1844). The corresponding DNA fragments were generated by polymerase chain reaction (PCR) and then cloned into pENTR/TEV/D-TOPO vector (Invitrogen). After being confirmed by sequencing, the right inserts were subcloned into destination vector of pHMGWA-His6-MBP by LR Clonase™ II enzyme mix (Invitrogen). The pHMGWA-His6-MBP vector is kindly provided by Dr. Busso, CNRS/INSERM/Université Louis Pasteur, France (30).

Filter Binding Assays

Filter binding assays of hDcr and different hDcr fragments were performed in the same way as previously described (7). Briefly, serial dilutions of hDcr protein were incubated in a buffer containing 20 mM Tris-HCl (pH 7.5), 25 mM NaCl, 5 mM EDTA, 1 mM dithiothreitol (DTT), 1% glycerol and ~0.5-1 nM (1500 CPM) of 5'-end ^{32}P-labeled duplex RNA substrate (one strand was labeled) at room temperature for 60 min in a 30 μl of total volume. Following incubation, a 25 μl aliquot of each reaction was applied to a dot-blot apparatus equipped with three membranes: Tuffryn, Protran and Nytran (from top to bottom). After drying, the bound (on Protran) or free (on Nytran) RNAs were quantified by a Phosphorimager (GE Healthcare). Percent bound RNA, calculated as the ratio of radioactivity detected on the Protran membrane over the total input radioactivity, was plotted as a function of protein concentration. K_d was determined by global fitting to the equation: $k_{obsd}=(k_{max}\times[\text{Dicer}])\,(K_d+[\text{Dicer}])-1$, where k_{obsd} is the observed rate constant at a given protein concentration, k_{max} is the maximal rate constant with saturating protein, and K_d is the protein concentration that provides half the maximal rate. Curve fitting was conducted with KaleidaGraph (Synergy Software, Reading, Pa.).

Dicing Assays

The cleavage assays of hDcr were carried out similarly as described previously (7). Simply, dsRNA substrates were 5'-end labeled with γ-^{32}P-ATP, annealed and incubated with 30 nM of hDcr (otherwise, stated in figure legends) at 37° C. for the specified time in a 10 μl volume (unless otherwise indicated) containing 20 mM Tris-HCl (pH 6.5), 1.5 mM $MgCl_2$, 25 mM NaCl, 1 mM DTT and 1% glycerol. Reactions were stopped by addition of 1.2 volumes of loading buffer (95% formamide, 18 mM EDTA, 0.025% SDS, 0.1% xylene cyanole FF and 0.1% bromophenol blue). After heating at 70° C. for 10 min, the samples were analyzed by electrophoresis through a 15% polyacrylamide-7M urea gel run in Tris-borate-EDTA (TBE) buffer and quantified using a Phosphorimager, and data quantification was achieved using ImageQuant TL.

ATPase Hydrolysis Assays

In vitro ATPase assay was performed as described elsewhere (31) with some modifications. ATPase hydrolysis assay was carried out in a 5 μl reaction of mixture containing 200 nM hDcr or ATPase/helicase domain, 200 nM dsRNA (or without RNA), 83.3 nM γ-^{32}P-ATP and 20 nM cold ATP in a buffer consisting of 50 mM MES (pH 6.5), 50 mM KAc, 2.5 mM $Mg(Ac)_2$, 1 mM dithiothreitol (DTT) and 0.1 mg/ml bovine serum albumin (BSA). The reaction mixture was incubated at 37° C. for the indicated time. After incubation, the reaction was terminated by addition of 2 μl of 50 mM EDTA. The reaction mixture was separated by loading 0.5 μl of the reaction mixture on the PEI-cellulose plate and running for ~1 hour in a buffer containing 0.5 M LiCl and 1 M formic acid. After drying, the polyethyleneimine (PEI)-cellulose plate was quantified using a Phosphorimager, and data quantification was achieved by using software of ImageQuant TL.

Pull-Down Assays

Six microgram of both of hTRBP2 and mbp-ATPase/hel-HA proteins were mixed with 15 μl of anti-hemagglutinin (anti-HA) antibody-coupled agarose beads in 1× phosphate-buffered saline (PBS) buffer (Sigma, Saint Louis, Mo.) and incubated in cold room and rocked 60 min. The mixture was pelleted by 30 sec spin at 10,000×g and then washed once with 1×PBS and followed by 5 times with the washing buffer of 20 mM Hepes (pH 7.5), 250 mM NaCl, 1% glycerol and 0.1% Triton X-100. After the last wash, the pellet was boiled for 3 min in 1.2×SDS protein loading buffer. As a control, hTRBP2 alone was also processed in the same way.

Results

A Fully Active hDcr can be Reconstituted from Trans-Expressed Fragments

The large size and multi-domain composition of hDcr have presented challenges to its expression, purification and analysis in recombinant form (4, 10). Previous studies have relied on the presence of endogenous hDcr in cell extracts or purified hDcr obtained by over-expression in baculovirus-infected insect cells. These approaches preclude ready analysis of hDcr domain functions due to the difficulties of preparing mutant proteins in these systems. Although prior attempts to express hDcr in *E. coli* were unsuccessful, it was reasoned that it might be possible to break the protein into smaller fragments that could be individually expressed in bacteria. Using full-length active recombinant hDcr purified from its baculovirus-infected Sf9 cells, limited proteolysis was performed using endoproteinase Glu-C to obtain globular hDcr fragments. This treatment produced two stable polypeptides (FIG. **1**A). The results from both mass spectrometry and Edman degradation sequencing showed that one fragment contains the ATPase/hel, DUF283, and PAZ domains (N-terminal fragment, hDcr-N) and the other contains the two tandem RNase III domains and the C-terminal dsRBD (C-terminal fragment, hDcr-C) (FIG. **1**A). Recombinant baculovirus constructs were prepared for these polypeptides and their expression was tested in baculovirus-infected Sf9 cells. Although the two fragments could not be individually expressed in this system, co-expression led to production of a stable complex (FIG. **1**B) that could not be disrupted by either 1 M sodium chloride or 4 M urea. To check whether the co-expressed complex was correctly folded and functional, cleavage assays were performed with a 35-base pair substrate (37ab, see FIG. **5**A). These dicing assays showed that the hDcr-N/C complex is active and its activity is similar to that of wild-type hDcr (FIG. **1**C, FIG. **6**).

FIGS. **1**A-C. Human Dicer can be Separated into Functional Fragments that Interact in Trans.

A. Proteolysis of full-length recombinant hDicer (FL-hDcr) protein. Dose-dependent proteolysis of FL-hDcr protein (10 μg for each reaction) with endoproteinase Glu-C was used to screen for optimal proteolytic conditions (left panel). The two identified globular protein fragments marked with hDcr-N and hDcr-C were isolated for mass spectrometry and Edman degradation sequencing. The isolated fragments of hDcr-N and hDcr-C from the partial proteolysis are represented in relation to wild-type FL-hDcr (right panel). B. Co-expression of the hDcr fragments in Sf9 cells. The co-expressed hDcr-N and hDcr-C fragments form a stable complex as shown from the elution profile of Superdex 200 size-exclusion chromatography (left panel). An SDS-PAGE gel shows the two protein fragments either from a Ni^{2+}-column after TEV protease cleavage (Ni^{2+}) or from the Superdex 200 size-exclusion column (Sup200). M is prestained protein ladder, SeeBlue Plus2 (Invitrogen). C. The complex (hDcr-N/hDcr-C) displays cleavage activity similar to that of FL-hDcr. In the cleavage assay, the hDcr-N/hDcr-C complex (lane 2) or FL-hDcr (lane 3) was incubated with 37ab RNA substrate, of which 37a was ^{32}P-labeled. From this substrate, hDcr generates two products of 22-nt and 15-nt.

FIG. **6**. The Activity of hDcr-N/C Complex Expressed in Trans is Similar to Wild-Type hDcr.

Time course dicing assays show no significant difference between trans-expressed hDcr-N/C complex and wild-type hDcr.

Direct Interaction of the PAZ and RNase III Domains Determines the Length of Dicer Products

The successful expression in trans of hDcr fragments in the baculovirus system encouraged us to further dissect hDcr using a bacterial expression system. It was tested whether the catalytic domains interact directly with the PAZ domain, an established RNA-binding motif that recognizes both the 5' and 3' ends at one terminus of a dsRNA (3, 6, 11-13). Based on published sequence alignment information (4, 10), hDcr-C was over-expressed in *E. coli* (FIG. **2**A). RNA cleavage assays showed that the dominant product of the purified hDcr-C fragment is 15-nts in length, in contrast to the characteristic 22-nt products generated by full-length Dicer (lanes 2-3, left panel, FIG. **2**B). For comparison, the main products generated by *E. coli* RNase III, a structural homolog of each of the RNase III domains of hDcr, are 12-nt in length (lane 4, left panel, FIG. **2**B). Another difference is that *E. coli* RNase III could cleave a 19 bp substrate, but the hDcr-C could not. Further cleavage assays showed that the hDcr-C protein can also cleave hairpin RNA, for example, pre-miR-20a, in a similar manner, generating a 15-nt product (middle and right panels, FIG. **2**B). To eliminate the possibility that this cleavage activity arises from RNase contamination during protein preparation, an hDcr-C protein variant containing point mutations in the two RNaseIII active sites (Glu1316Ala and Glu1705A1a) was expressed. These mutations abolished cleavage activity (lane 5, left panel, FIG. **2**B).

To assess the role of the PAZ domain in determining Dicer cleavage product length, an attempt was made to express the PAZ domain alone in *E. coli*. Although this was unsuccessful, a construct including both the PAZ domain and the adjacent DUF283 region yielded soluble protein (hereafter the tandem construct named DP, FIG. **2**A). RNA cleavage assays showed that addition of the DP polypeptide to the hDcr-C cleavage reaction led to RNA products similar to those produced by full-length Dicer (lanes 6 and 7, FIG. **2**C), indicating that DP and hDcr-C proteins are correctly folded and interact with each other. To test for a direct protein-protein interaction, hDcr-C and DP polypeptides were incubated together in the absence of RNA and then analyzed by size exclusion chromatography. The elution profile indicated that DP and hDcr-C form a stable complex (FIG. **7**). Addition of the ATPase/Helicase domain (further discussed in FIG. **4**, **5**) in the cleavage reactions did not affect the cleavage pattern (lanes 3-4 or lanes 6-7, FIG. **2**C). The fact that the PAZ domain binds 7-nt-long dsRNA (12, 13) and the hDcr-C generates 15-nt products suggests that the size of hDcr products (22-nt) is determined by the combined footprints of the PAZ and RNase III domains on the RNA.

FIGS. **2**A-C.

Cooperative action between the PAZ and RNase III domains determines the size of hDcr products. A. Schematic representation of the bacterially expressed tandem DUF283 and PAZ domains (DP) and hDcr-C. B. Cleavage assays with hDcr-C. hDcr-C mainly generates 15-nt products from a dsRNA (lane 3), while *E. coli* RNase III gives 12-nt products (lane 4). As a negative control, hDcr-C with mutations in the active site glutamines (1316(E/A) and 1705(E/A)) in the RNase III domains (mthDcr-C) displayed no activity (lane 5). Middle and right panels are the cleavage assays of hDcr-C on a dsRNA (37ab) and a pre-microRNA (pre-miR-20a). In both cases, hDcr-C mainly generates a 15-nt product. C. PAZ and RNase III domains together determine the size of hDcr product. Addition of the middle domains of hDcr (DP) to the cleavage reaction (lane 6-7) restored dicing patterns displayed by FL-hDcr (compare lanes 2-3 to lanes 6-7). ATPase/hel domain played no role in cleavage activity (compare lane 4 to lane 5, or lane 6 to lane 7). FL-hDcr (lane 2) and hDcr-N/hDcr-C complex (lane 3) were used as positive controls, which generate the 22-nt and 15-nt products. The RNA substrate used in these assays was 37ab RNA, of which 37a was 5'-^{32}P-labeled.

FIG. **7**.

DP forms a stable complex with hDcr-C. A pre-incubated mixture of the hDcr-C fragment with 3-fold excess of DP was analyzed with a Superdex 200 size-exclusion column (top panel, elution profile). SDS/PAGE analysis of the Superdex 200 fractions indicates that both proteins are present in the first peak and the excess DP elutes in the second peak (bottom panel).

The C-Terminal dsRBD is Required for RNA Substrate Binding and Cleavage Activities of hDcr-C

It has been reported that the dsRBD of *E. coli* RNase III is not required for substrate cleavage (14), while this domain is necessary for the activity of human *Drosha*, another RNase III family enzyme in the microRNA pathway (8). To assess the importance of the C-terminal dsRBD in the hDcr-C construct, the hDcr-C lacking this dsRBD was expressed (hDcr-CΔRBD, FIG. **3**A). The analysis showed that the hDcr-CΔRBD protein alone had no cleavage activity (lanes 1-2, FIG. **3**B), indicating that the terminal dsRBD could be necessary for hDcr-C to bind or cleave dsRNA. To test whether the bacteria-expressed hDcr-CΔRBD retains its native fold and catalytic capability, dsRNA cleavage assays were performed by addition of the DP polypeptide to the cleavage reactions. These assays showed that the presence of DP restored the dicing pattern of hDcr (lanes 3-4, FIG. **3**B). It was also found that deletion of the dsRBD from hDcr-C did not affect the complex formation of the hDcr-CΔRBD with DP. Therefore, the terminal dsRBD is necessary for substrate cleavage by the hDcr-C fragment, but does not affect the folding or catalytic function of the RNaseIII domains.

FIGS. **3**A and **3**B. The C-Terminal dsRBD is Required for RNA Binding and Cleavage in the Absence of the PAZ Domain.

A. Schematic representation of bacterially expressed hDcr-C without the C-terminal dsRBD (hDcr-CΔRBD). B. Requirement of dsRBD for the cleavage activity of hDcr-C. Deletion of dsRBD from hDcr-C fragment abolishes its substrate cleavage activity (lane 1-2). Addition of the middle domains of hDcr (DP) into the cleavage reactions restored FL-hDcr cleavage pattern (lanes 3-4). The ATPase/helicase domain played no role in the cleavage activity (compare lane 1 to lane 2, or lane 3 to lane 4).

To establish the relationship between cleavage activity and substrate binding, nitrocellulose filter-binding assays were performed with three kinds of RNAs under non-cleavage conditions: substrate dsRNA (37 ab), Dicer product-mimic dsRNA (19-bp) and a pre-miRNA (pre-hlet-7a-1). The DP fragment bound more strongly to perfectly matched dsRNAs (either substrate or product RNAs) than to the hairpin pre-miRNA (K_d~200 nM versus ~1 μM, Table 1). By contrast, the hDcr-C fragment bound with measurable affinity only to the substrate dsRNA (K_d~300 nM) and displayed almost no binding to either the hairpin or product RNAs (Table 1). These RNA binding data are consistent with the above cleavage results showing that the hDcr-C protein is more active towards long, perfectly matched dsRNA substrates relative to pre-miRNAs. Removal of the terminal dsRBD domain from hDcr-C abolishes its RNA binding ability, indicating that this domain is required for the binding activity of hDcr to dsRNA in the absence of PAZ domain (Table 1).

TABLE 1

K_D values (nM) for human Dicer proteins*			
RNA substrate	pre-hlet7a-1	37ab	21ab
FL-hDicer	39 ± 5	53 ± 8	144 ± 23
mbp-ATPase/hel	96 ± 10	476 ± 30	n.d.
DP	~1000	200 ± 34	220 ± 40
hDcr C	n.d.	300 ± 40	n.d.
hDcr CΔRBD	n.d.	n.d.	n.d.

*n.d. = out of the detectable limit

The hDcr ATPase/Hel Domain is Important for Substrate Selectivity Towards Pre-miRNAs

Based on our previous results, it was concluded that the C-terminal hDcr fragment binds and cleaves perfect duplexes preferentially over hairpin RNAs (FIG. **2**B). However, wild-type hDcr prefers to bind and cleave hairpin RNAs (7, 9). It was hypothesized that the hDcr-N polypeptide, which includes the ATPase/hel, DUF, and PAZ domains, might play a role in pre-miRNA processing. Although this fragment could not be expressed on its own either in insect cells or in *E. coli*, a construct containing the complete ATPase/helicase domain of hDcr fused with maltose-binding protein (MBP) that could be produced in *E. coli* was identified (FIG. **4**A).

Since hDcr interacts with human TAR-RNA binding protein (hTRBP2) via its helicase domain (15-18), whether the MBP-ATPase/hel fusion retains the ability to bind to the recombinant hTRBP2 was tested. Both size exclusion chromatography and co-immunoprecipitation assays showed that the helicase domain interacts with hTRBP2 (FIG. **4**B, C), indicating that the purified MBP-ATPase/hel protein is likely to be correctly folded. Furthermore, ATP hydrolysis assays showed that the ATPase/hel domain of hDcr retained its ability to hydrolyze ATP in vitro. FIG. **8**.

It was previously demonstrated that wild-type hDcr prefers to cleave the pre-hlet7a-1 RNA relative to a perfectly matched duplex RNA substrate (7, 9). Furthermore, it has also been reported that the ATPase/hel domain is involved in the production of siRNAs from long dsRNA substrates (19, 20). To further understand the role of the helicase domain in the processing of RNA substrates, the substrate binding properties of the MBP-ATPase/hel protein were studied using filter binding assays. The helicase domain prefers to bind to the pre-hlet-7a-1 substrate with a K_d of ~100 nM for the hairpin RNA. In contrast, the helicase domain bound the 37ab RNA with a Kd of ~500 nM, while it did not bind appreciably to a 21 nt RNA (Table 1).

FIGS. **4**A-C.

ATPase/Helicase domain of hDcr interacts with TRBP. A. Schematic representation of bacterially expressed ATPase/hel domain tagged with MBP. B. The interaction of MBP-ATPase/hel fragment with hTRBP2. A pre-incubated mixture of the MBP-ATPase/hel fragment with 3-fold excess of hTRBP2 was fractionated with a Superdex 200 size-exclusion column (top panel, elution profile). SDS/PAGE gel analysis of the Superdex 200 fractions indicates that MBP-ATPase/hel and hTRBP2 interact as shown in the first peak (bottom panel). The excess hTRBP2 elutes in the second peak. C. MBP-ATPase/hel can pull-down hTRBP2. The MBP-ATPase/hel-domain was purified with a C-terminal hemagglutinin (HA) epitope tag. The two purified proteins (30 pmol of hDcr and 130 pmol of hTRBP2) were incubated on ice with anti-HA antibody agarose beads (Sigma-Aldrich) for 60 min prior to several washes. The bound proteins are eluted via boiling with 1.2×SDS buffer. HC is the antibody heavy chain, while the light chain was run out. M is prestained protein ladder, SeeBlue Plus2 (Invitrogen).

FIG. **8**. ATPase Activity of FL-hDcr and MBP-ATPase/Hel.

Quantitation of ATPase activities of FL-hDcr and MBP-ATPase/Hel are determined via TLC analyses. The ATPase activity of FL-hDcr can be moderately stimulated by dsRNA (left panel), while the activity of MBP-ATPase/Hel is not (right panel).

The preferred binding of the helicase domain to pre-hlet-7a-1 may reflect the existence of an interaction between the helicase domain and the terminal loop, and this interaction may play an important role in the selection of this type of RNA substrate by hDcr. To test this possibility, a hairpin RNA (37ab-loop, FIG. **5**A) was designed, containing the perfectly matched stem derived from the 37ab RNA substrate (a slow-cleavable RNA) and the terminal loop from pre-hlet-7a-1 (a fast-cleavable RNA). Dicing assays showed that hDcr cleaves the 37ab-loop substrate with a rate similar to that observed for the wild-type pre-hlet-7a-1 RNA (FIG. **5**B, **5**C). Specifically, under single-turnover conditions, the time required to cleave 50% of the labeled substrate ($t_{1/2}$) was approximately 1 min, 3 min, and 65 min for pre-hlet-7a-1, 37ab-loop, and 37ab, respectively (left panel, FIG. **5**C). Furthermore, a bulged substrate RNA (hlet7-stem) that is derived from pre-hlet7a-1 became an unfavorable substrate, with a cleavage pattern similar to the 37 ab RNA substrate (left panel, FIG. **5**C). In addition, the hDcr without the helicase domain, however, hydrolyzed all of the substrates (perfectly matched or bulged dsRNA, or pre-miRNA) in a similar manner (right panel, FIG. **5**C). Taken together with above binding data, these results suggest that the ATPase/hel domain plays the role of a "gate-keeper" in order to screen RNA substrates and that its interaction with the terminal loop, not the bulged stem, regulates the dicing activity of hDcr on pre-hlet-7a-1.

FIGS. **5**A and **5**B.

Terminal loop of pre-hlet-7a-1 determines the substrate selection by interacting with the ATPase/helicase domain. A. Schematic representation of four RNA substrates: pre-hlet-7a-1 is abbreviated from human pre-let-7a-1; hlet7-stem is constructed from pre-hlet-7a-1 stem plus an additional 15 bps; 37ab represents a pre-siRNA; and 37ab-loop is an artificial hairpin RNA made of the 37ab stem and the terminal loop from pre-hlet-7a-1. The perfect base pairs are depicted with vertical lines in the cartoon, while G-U wobbles are marked with dots. The terminal loop structure is predicted from MFOLD and marked with grey color. B. Actual cleavage images of a natural hair RNA (pre-hlet-7a-1) and an artificial hairpin RNA (37ab-loop). These two hairpin RNAs have same terminal loop and they were cleaved similarly by wild-type hDcr. C. Interaction of terminal loop with ATPase/helicase domain determines processing activity of hDcr. The top panels show images of dicing reactions from natural pre-hlet-7a-1 and an artificial hairpin RNA, 37ab-loop. The bottom panels (from left to right) are the quantitation of dicing assays from FL-hDcr and hDcr without ATPase/hel domain on the RNA substrates shown in A.

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While the present invention has been described with reference to the specific embodiments thereof, it should be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt a particular situation, material, composition of matter, process, process step or steps, to the objective, spirit and scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

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Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
                165                 170                 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
            180                 185                 190

Cys Glu Asn Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
        195                 200                 205

Ile Leu Asn Gly Lys Cys Asp Pro Glu Glu Leu Glu Glu Lys Ile Gln
    210                 215                 220

Lys Leu Glu Lys Ile Leu Lys Ser Asn Ala Glu Thr Ala Thr Asp Leu
225                 230                 235                 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
                245                 250                 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Glu Arg Leu Leu Met
            260                 265                 270

Glu Leu Glu Glu Ala Leu Asn Phe Ile Asn Asp Cys Asn Ile Ser Val
        275                 280                 285

His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
    290                 295                 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305                 310                 315                 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
                325                 330                 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Phe Leu
            340                 345                 350

Arg Lys Ile His Ala Leu Cys Glu Glu His Phe Ser Pro Ala Ser Leu
        355                 360                 365

Asp Leu Lys Phe Val Thr Pro Lys Val Ile Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Glu Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
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        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Asn Lys Gln Met Glu Ala
                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Ile Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Ile Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Val Asp Thr Gly Glu Thr Asp Ile Asp Pro Val
        595                 600


<210> SEQ ID NO 3
<211> LENGTH: 1318
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 3

Met Asp Asp Asp Asp Val Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp
1               5                   10                  15

Gly Gly Pro Arg Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg
            20                  25                  30

Tyr Cys Ala Arg Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys
        35                  40                  45

Cys Arg Thr Arg Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr
    50                  55                  60

Leu Pro Ile Asn Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met
65                  70                  75                  80

Ser Cys Val Arg Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu
                85                  90                  95

Lys Leu His Lys Ile Gly Glu Leu Asp Asp His Leu Met Pro Val Gly
            100                 105                 110

Lys Glu Thr Val Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu
        115                 120                 125

Glu Thr Ser Val Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys
    130                 135                 140

Tyr Pro Lys Ala Ile Pro Glu Cys Leu Arg Asp Ser Tyr Pro Arg Pro
145                 150                 155                 160

Asp Gln Pro Cys Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro
                165                 170                 175

Leu Pro Asp Glu Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu
            180                 185                 190

Asp Thr Thr Arg Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln
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        195                 200                 205

Ile Pro His Phe Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser
    210                 215                 220

Ile Glu Leu Lys Lys Ser Gly Phe Met Leu Ser Leu Gln Met Leu Glu
225                 230                 235                 240

Leu Ile Thr Arg Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu
                245                 250                 255

Glu Lys Pro Ala Leu Glu Phe Lys Pro Thr Asp Ala Asp Ser Ala Tyr
            260                 265                 270

Cys Val Leu Pro Leu Asn Val Val Asn Asp Ser Ser Thr Leu Asp Ile
        275                 280                 285

Asp Phe Lys Phe Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly
    290                 295                 300

Ile Pro Ser Thr Lys Tyr Thr Lys Glu Thr Pro Phe Val Phe Lys Leu
305                 310                 315                 320

Glu Asp Tyr Gln Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp
                325                 330                 335

Gln Pro His Arg Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro
            340                 345                 350

Leu Ser Lys Phe Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr
        355                 360                 365

Lys Thr Lys Tyr Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu
    370                 375                 380

Asp Val Asp His Thr Ser Ser Arg Leu Asn Leu Leu Thr Pro Arg His
385                 390                 395                 400

Leu Asn Gln Lys Gly Lys Ala Leu Pro Leu Ser Ser Ala Glu Lys Arg
                405                 410                 415

Lys Ala Lys Trp Glu Ser Leu Gln Asn Lys Gln Ile Leu Val Pro Glu
            420                 425                 430

Leu Cys Ala Ile His Pro Ile Pro Ala Ser Leu Trp Arg Lys Ala Val
        435                 440                 445

Cys Leu Pro Ser Ile Leu Tyr Arg Leu His Cys Leu Leu Thr Ala Glu
    450                 455                 460

Glu Leu Arg Ala Gln Thr Ala Ser Asp Ala Gly Val Gly Val Arg Ser
465                 470                 475                 480

Leu Pro Ala Asp Phe Arg Tyr Pro Asn Leu Asp Phe Gly Trp Lys Lys
                485                 490                 495

Ser Ile Asp Ser Lys Ser Phe Ile Ser Ile Ser Asn Ser Ser Ser Ala
            500                 505                 510

Glu Asn Asp Asn Tyr Cys Lys His Ser Thr Ile Val Pro Glu Asn Ala
        515                 520                 525

Ala His Gln Gly Ala Asn Arg Thr Ser Ser Leu Glu Asn His Asp Gln
    530                 535                 540

Met Ser Val Asn Cys Arg Thr Leu Leu Ser Glu Ser Pro Gly Lys Leu
545                 550                 555                 560

His Val Glu Val Ser Ala Asp Leu Thr Ala Ile Asn Gly Leu Ser Tyr
                565                 570                 575

Asn Gln Asn Leu Ala Asn Gly Ser Tyr Asp Leu Ala Asn Arg Asp Phe
            580                 585                 590

Cys Gln Gly Asn Gln Leu Asn Tyr Tyr Lys Gln Glu Ile Pro Val Gln
        595                 600                 605

Pro Thr Thr Ser Tyr Ser Ile Gln Asn Leu Tyr Ser Tyr Glu Asn Gln
    610                 615                 620
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Pro Gln Pro Ser Asp Glu Cys Thr Leu Leu Ser Asn Lys Tyr Leu Asp
625                 630                 635                 640

Gly Asn Ala Asn Lys Ser Thr Ser Asp Gly Ser Pro Val Met Ala Val
                645                 650                 655

Met Pro Gly Thr Thr Asp Thr Ile Gln Val Leu Lys Gly Arg Met Asp
            660                 665                 670

Ser Glu Gln Ser Pro Ser Ile Gly Tyr Ser Ser Arg Thr Leu Gly Pro
        675                 680                 685

Asn Pro Gly Leu Ile Leu Gln Ala Leu Thr Leu Ser Asn Ala Ser Asp
    690                 695                 700

Gly Phe Asn Leu Glu Arg Leu Glu Met Leu Gly Asp Ser Phe Leu Lys
705                 710                 715                 720

His Ala Ile Thr Thr Tyr Leu Phe Cys Thr Tyr Pro Asp Ala His Glu
                725                 730                 735

Gly Arg Leu Ser Tyr Met Arg Ser Lys Lys Val Ser Asn Cys Asn Leu
            740                 745                 750

Tyr Arg Leu Gly Lys Lys Lys Gly Leu Pro Ser Arg Met Val Val Ser
        755                 760                 765

Ile Phe Asp Pro Pro Val Asn Trp Leu Pro Pro Gly Tyr Val Val Asn
    770                 775                 780

Gln Asp Lys Ser Asn Thr Asp Lys Trp Glu Lys Asp Glu Met Thr Lys
785                 790                 795                 800

Asp Cys Met Leu Ala Asn Gly Lys Leu Asp Glu Asp Tyr Glu Glu Glu
                805                 810                 815

Asp Glu Glu Glu Glu Ser Leu Met Trp Arg Ala Pro Lys Glu Glu Ala
            820                 825                 830

Asp Tyr Glu Asp Asp Phe Leu Glu Tyr Asp Gln Glu His Ile Arg Phe
        835                 840                 845

Ile Asp Asn Met Leu Met Gly Ser Gly Ala Phe Val Lys Lys Ile Ser
    850                 855                 860

Leu Ser Pro Phe Ser Thr Thr Asp Ser Ala Tyr Glu Trp Lys Met Pro
865                 870                 875                 880

Lys Lys Ser Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe Glu Asp
                885                 890                 895

Phe Asp Tyr Ser Ser Trp Asp Ala Met Cys Tyr Leu Asp Pro Ser Lys
            900                 905                 910

Ala Val Glu Glu Asp Asp Phe Val Val Gly Phe Trp Asn Pro Ser Glu
        915                 920                 925

Glu Asn Cys Gly Val Asp Thr Gly Lys Gln Ser Ile Ser Tyr Asp Leu
    930                 935                 940

His Thr Glu Gln Cys Ile Ala Asp Lys Ser Ile Ala Asp Cys Val Glu
945                 950                 955                 960

Ala Leu Leu Gly Cys Tyr Leu Thr Ser Cys Gly Glu Arg Ala Ala Gln
                965                 970                 975

Leu Phe Leu Cys Ser Leu Gly Leu Lys Val Leu Pro Val Ile Lys Arg
            980                 985                 990

Thr Asp Arg Glu Lys Ala Leu Cys  Pro Thr Arg Glu Asn  Phe Asn Ser
        995                 1000                1005

Gln Gln  Lys Asn Leu Ser Val  Ser Cys Ala Ala Ala  Ser Val Ala
    1010                1015                1020

Ser Ser  Arg Ser Ser Val Leu  Lys Asp Ser Glu Tyr  Gly Cys Leu
    1025                1030                1035
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Lys Ile  Pro Pro Arg Cys Met  Phe Asp His Pro Asp  Ala Asp Lys
    1040                 1045                 1050

Thr Leu  Asn His Leu Ile Ser  Gly Phe Glu Asn Phe  Glu Lys Lys
    1055                 1060                 1065

Ile Asn  Tyr Arg Phe Lys Asn  Lys Ala Tyr Leu Leu  Gln Ala Phe
    1070                 1075                 1080

Thr His  Ala Ser Tyr His Tyr  Asn Thr Ile Thr Asp  Cys Tyr Gln
    1085                 1090                 1095

Arg Leu  Glu Phe Leu Gly Asp  Ala Ile Leu Asp Tyr  Leu Ile Thr
    1100                 1105                 1110

Lys His  Leu Tyr Glu Asp Pro  Arg Gln His Ser Pro  Gly Val Leu
    1115                 1120                 1125

Thr Asp  Leu Arg Ser Ala Leu  Val Asn Asn Thr Ile  Phe Ala Ser
    1130                 1135                 1140

Leu Ala  Val Lys Tyr Asp Tyr  His Lys Tyr Phe Lys  Ala Val Ser
    1145                 1150                 1155

Pro Glu  Leu Phe His Val Ile  Asp Asp Phe Val Gln  Phe Gln Leu
    1160                 1165                 1170

Glu Lys  Asn Glu Met Gln Gly  Met Asp Ser Glu Leu  Arg Arg Ser
    1175                 1180                 1185

Glu Glu  Asp Glu Glu Lys Glu  Glu Asp Ile Glu Val  Pro Lys Ala
    1190                 1195                 1200

Met Gly  Asp Ile Phe Glu Ser  Leu Ala Gly Ala Ile  Tyr Met Asp
    1205                 1210                 1215

Ser Gly  Met Ser Leu Glu Thr  Val Trp Gln Val Tyr  Tyr Pro Met
    1220                 1225                 1230

Met Arg  Pro Leu Ile Glu Lys  Phe Ser Ala Asn Val  Pro Arg Ser
    1235                 1240                 1245

Pro Val  Arg Glu Leu Leu Glu  Met Glu Pro Glu Thr  Ala Lys Phe
    1250                 1255                 1260

Ser Pro  Ala Glu Arg Thr Tyr  Asp Gly Lys Val Arg  Val Thr Val
    1265                 1270                 1275

Glu Val  Val Gly Lys Gly Lys  Phe Lys Gly Val Gly  Arg Ser Tyr
    1280                 1285                 1290

Arg Ile  Ala Lys Ser Ala Ala  Ala Arg Arg Ala Leu  Arg Ser Leu
    1295                 1300                 1305

Lys Ala  Asn Gln Pro Gln Val  Pro Asn Ser
    1310                 1315


<210> SEQ ID NO 4
<211> LENGTH: 1922
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 4

Met Lys Ser Pro Ala Leu Gln Pro Leu Ser Met Ala Gly Leu Gln Leu
1               5                   10                  15

Met Thr Pro Ala Ser Ser Pro Met Gly Pro Phe Phe Gly Leu Pro Trp
            20                  25                  30

Gln Gln Glu Ala Ile His Asp Asn Ile Tyr Thr Pro Arg Lys Tyr Gln
        35                  40                  45

Val Glu Leu Leu Glu Ala Ala Leu Asp His Asn Thr Ile Val Cys Leu
    50                  55                  60
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Asn Thr Gly Ser Gly Ala Thr Phe Ile Ala Val Leu Leu Thr Lys Glu
65                  70                  75                  80

Leu Ser Tyr Gln Ile Arg Gly Asp Phe Ser Arg Asn Gly Lys Arg Thr
                85                  90                  95

Val Phe Leu Val Asn Ser Ala Asn Gln Val Ala Gln Gln Val Ser Ala
            100                 105                 110

Val Arg Thr His Ser Asp Leu Lys Val Gly Glu Tyr Ser Asn Leu Glu
        115                 120                 125

Val Asn Ala Ser Trp Thr Lys Glu Arg Trp Asn Gln Glu Phe Thr Lys
    130                 135                 140

His Gln Val Leu Ile Met Thr Cys Tyr Val Ala Leu Asn Val Leu Lys
145                 150                 155                 160

Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
                165                 170                 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
            180                 185                 190

Cys Glu Asn Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
        195                 200                 205

Ile Leu Asn Gly Lys Cys Asp Pro Glu Glu Leu Glu Glu Lys Ile Gln
    210                 215                 220

Lys Leu Glu Lys Ile Leu Lys Ser Asn Ala Glu Thr Ala Thr Asp Leu
225                 230                 235                 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
                245                 250                 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Glu Arg Leu Leu Met
            260                 265                 270

Glu Leu Glu Glu Ala Leu Asn Phe Ile Asn Asp Cys Asn Ile Ser Val
        275                 280                 285

His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
    290                 295                 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305                 310                 315                 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
                325                 330                 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Phe Leu
            340                 345                 350

Arg Lys Ile His Ala Leu Cys Glu Glu His Phe Ser Pro Ala Ser Leu
        355                 360                 365

Asp Leu Lys Phe Val Thr Pro Lys Val Ile Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Glu Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Asn Lys Gln Met Glu Ala
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                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Ile Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Ile Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Val Asp Thr Gly Glu Thr Asp Ile Asp Pro Val Met Asp Asp Asp
        595                 600                 605

Asp Val Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp Gly Gly Pro Arg
    610                 615                 620

Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg Tyr Cys Ala Arg
625                 630                 635                 640

Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys Cys Arg Thr Arg
                645                 650                 655

Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr Leu Pro Ile Asn
            660                 665                 670

Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met Ser Cys Val Arg
        675                 680                 685

Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu Lys Leu His Lys
    690                 695                 700

Ile Gly Glu Leu Asp Asp His Leu Met Pro Val Gly Lys Glu Thr Val
705                 710                 715                 720

Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu Glu Thr Ser Val
                725                 730                 735

Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys Tyr Pro Lys Ala
            740                 745                 750

Ile Pro Glu Cys Leu Arg Asp Ser Tyr Pro Arg Pro Asp Gln Pro Cys
        755                 760                 765

Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro Leu Pro Asp Glu
    770                 775                 780

Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu Asp Thr Thr Arg
785                 790                 795                 800

Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln Ile Pro His Phe
                805                 810                 815

Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser Ile Glu Leu Lys
            820                 825                 830

Lys Ser Gly Phe Met Leu Ser Leu Gln Met Leu Glu Leu Ile Thr Arg
        835                 840                 845

Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu Glu Lys Pro Ala
    850                 855                 860

Leu Glu Phe Lys Pro Thr Asp Ala Asp Ser Ala Tyr Cys Val Leu Pro
865                 870                 875                 880

Leu Asn Val Val Asn Asp Ser Ser Thr Leu Asp Ile Asp Phe Lys Phe
                885                 890                 895

Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly Ile Pro Ser Thr
            900                 905                 910
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Lys Tyr Thr Lys Glu Thr Pro Phe Val Phe Lys Leu Glu Asp Tyr Gln
        915                 920                 925

Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp Gln Pro His Arg
    930                 935                 940

Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro Leu Ser Lys Phe
945                 950                 955                 960

Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr Lys Thr Lys Tyr
                965                 970                 975

Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu Asp Val Asp His
            980                 985                 990

Thr Ser Ser Arg Leu Asn Leu Leu  Thr Pro Arg His Leu  Asn Gln Lys
        995                 1000                1005

Gly Lys  Ala Leu Pro Leu Ser  Ser Ala Glu Lys Arg  Lys Ala Lys
    1010                1015                1020

Trp Glu  Ser Leu Gln Asn Lys  Gln Ile Leu Val Pro  Glu Leu Cys
    1025                1030                1035

Ala Ile  His Pro Ile Pro Ala  Ser Leu Trp Arg Lys  Ala Val Cys
    1040                1045                1050

Leu Pro  Ser Ile Leu Tyr Arg  Leu His Cys Leu Leu  Thr Ala Glu
    1055                1060                1065

Glu Leu  Arg Ala Gln Thr Ala  Ser Asp Ala Gly Val  Gly Val Arg
    1070                1075                1080

Ser Leu  Pro Ala Asp Phe Arg  Tyr Pro Asn Leu Asp  Phe Gly Trp
    1085                1090                1095

Lys Lys  Ser Ile Asp Ser Lys  Ser Phe Ile Ser Ile  Ser Asn Ser
    1100                1105                1110

Ser Ser  Ala Glu Asn Asp Asn  Tyr Cys Lys His Ser  Thr Ile Val
    1115                1120                1125

Pro Glu  Asn Ala Ala His Gln  Gly Ala Asn Arg Thr  Ser Ser Leu
    1130                1135                1140

Glu Asn  His Asp Gln Met Ser  Val Asn Cys Arg Thr  Leu Leu Ser
    1145                1150                1155

Glu Ser  Pro Gly Lys Leu His  Val Glu Val Ser Ala  Asp Leu Thr
    1160                1165                1170

Ala Ile  Asn Gly Leu Ser Tyr  Asn Gln Asn Leu Ala  Asn Gly Ser
    1175                1180                1185

Tyr Asp  Leu Ala Asn Arg Asp  Phe Cys Gln Gly Asn  Gln Leu Asn
    1190                1195                1200

Tyr Tyr  Lys Gln Glu Ile Pro  Val Gln Pro Thr Thr  Ser Tyr Ser
    1205                1210                1215

Ile Gln  Asn Leu Tyr Ser Tyr  Glu Asn Gln Pro Gln  Pro Ser Asp
    1220                1225                1230

Glu Cys  Thr Leu Leu Ser Asn  Lys Tyr Leu Asp Gly  Asn Ala Asn
    1235                1240                1245

Lys Ser  Thr Ser Asp Gly Ser  Pro Val Met Ala Val  Met Pro Gly
    1250                1255                1260

Thr Thr  Asp Thr Ile Gln Val  Leu Lys Gly Arg Met  Asp Ser Glu
    1265                1270                1275

Gln Ser  Pro Ser Ile Gly Tyr  Ser Ser Arg Thr Leu  Gly Pro Asn
    1280                1285                1290

Pro Gly  Leu Ile Leu Gln Ala  Leu Thr Leu Ser Asn  Ala Ser Asp
    1295                1300                1305
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Gly Phe  Asn Leu Glu Arg Leu  Glu Met Leu Gly Asp  Ser Phe Leu
    1310                 1315                 1320

Lys His  Ala Ile Thr Thr Tyr  Leu Phe Cys Thr Tyr  Pro Asp Ala
    1325                 1330                 1335

His Glu  Gly Arg Leu Ser Tyr  Met Arg Ser Lys Lys  Val Ser Asn
    1340                 1345                 1350

Cys Asn  Leu Tyr Arg Leu Gly  Lys Lys Lys Gly Leu  Pro Ser Arg
    1355                 1360                 1365

Met Val  Val Ser Ile Phe Asp  Pro Pro Val Asn Trp  Leu Pro Pro
    1370                 1375                 1380

Gly Tyr  Val Val Asn Gln Asp  Lys Ser Asn Thr Asp  Lys Trp Glu
    1385                 1390                 1395

Lys Asp  Glu Met Thr Lys Asp  Cys Met Leu Ala Asn  Gly Lys Leu
    1400                 1405                 1410

Asp Glu  Asp Tyr Glu Glu Glu  Asp Glu Glu Glu Glu  Ser Leu Met
    1415                 1420                 1425

Trp Arg  Ala Pro Lys Glu Glu  Ala Asp Tyr Glu Asp  Asp Phe Leu
    1430                 1435                 1440

Glu Tyr  Asp Gln Glu His Ile  Arg Phe Ile Asp Asn  Met Leu Met
    1445                 1450                 1455

Gly Ser  Gly Ala Phe Val Lys  Lys Ile Ser Leu Ser  Pro Phe Ser
    1460                 1465                 1470

Thr Thr  Asp Ser Ala Tyr Glu  Trp Lys Met Pro Lys  Lys Ser Ser
    1475                 1480                 1485

Leu Gly  Ser Met Pro Phe Ser  Ser Asp Phe Glu Asp  Phe Asp Tyr
    1490                 1495                 1500

Ser Ser  Trp Asp Ala Met Cys  Tyr Leu Asp Pro Ser  Lys Ala Val
    1505                 1510                 1515

Glu Glu  Asp Asp Phe Val Val  Gly Phe Trp Asn Pro  Ser Glu Glu
    1520                 1525                 1530

Asn Cys  Gly Val Asp Thr Gly  Lys Gln Ser Ile Ser  Tyr Asp Leu
    1535                 1540                 1545

His Thr  Glu Gln Cys Ile Ala  Asp Lys Ser Ile Ala  Asp Cys Val
    1550                 1555                 1560

Glu Ala  Leu Leu Gly Cys Tyr  Leu Thr Ser Cys Gly  Glu Arg Ala
    1565                 1570                 1575

Ala Gln  Leu Phe Leu Cys Ser  Leu Gly Leu Lys Val  Leu Pro Val
    1580                 1585                 1590

Ile Lys  Arg Thr Asp Arg Glu  Lys Ala Leu Cys Pro  Thr Arg Glu
    1595                 1600                 1605

Asn Phe  Asn Ser Gln Gln Lys  Asn Leu Ser Val Ser  Cys Ala Ala
    1610                 1615                 1620

Ala Ser  Val Ala Ser Ser Arg  Ser Ser Val Leu Lys  Asp Ser Glu
    1625                 1630                 1635

Tyr Gly  Cys Leu Lys Ile Pro  Pro Arg Cys Met Phe  Asp His Pro
    1640                 1645                 1650

Asp Ala  Asp Lys Thr Leu Asn  His Leu Ile Ser Gly  Phe Glu Asn
    1655                 1660                 1665

Phe Glu  Lys Lys Ile Asn Tyr  Arg Phe Lys Asn Lys  Ala Tyr Leu
    1670                 1675                 1680

Leu Gln  Ala Phe Thr His Ala  Ser Tyr His Tyr Asn  Thr Ile Thr
    1685                 1690                 1695

Asp Cys  Tyr Gln Arg Leu Glu  Phe Leu Gly Asp Ala  Ile Leu Asp
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    1700                1705                1710

Tyr Leu  Ile Thr Lys His Leu  Tyr Glu Asp Pro Arg  Gln His Ser
    1715                1720                1725

Pro Gly  Val Leu Thr Asp Leu  Arg Ser Ala Leu Val  Asn Asn Thr
    1730                1735                1740

Ile Phe  Ala Ser Leu Ala Val  Lys Tyr Asp Tyr His  Lys Tyr Phe
    1745                1750                1755

Lys Ala  Val Ser Pro Glu Leu  Phe His Val Ile Asp  Asp Phe Val
    1760                1765                1770

Gln Phe  Gln Leu Glu Lys Asn  Glu Met Gln Gly Met  Asp Ser Glu
    1775                1780                1785

Leu Arg  Arg Ser Glu Glu Asp  Glu Glu Lys Glu Glu  Asp Ile Glu
    1790                1795                1800

Val Pro  Lys Ala Met Gly Asp  Ile Phe Glu Ser Leu  Ala Gly Ala
    1805                1810                1815

Ile Tyr  Met Asp Ser Gly Met  Ser Leu Glu Thr Val  Trp Gln Val
    1820                1825                1830

Tyr Tyr  Pro Met Met Arg Pro  Leu Ile Glu Lys Phe  Ser Ala Asn
    1835                1840                1845

Val Pro  Arg Ser Pro Val Arg  Glu Leu Leu Glu Met  Glu Pro Glu
    1850                1855                1860

Thr Ala  Lys Phe Ser Pro Ala  Glu Arg Thr Tyr Asp  Gly Lys Val
    1865                1870                1875

Arg Val  Thr Val Glu Val Val  Gly Lys Gly Lys Phe  Lys Gly Val
    1880                1885                1890

Gly Arg  Ser Tyr Arg Ile Ala  Lys Ser Ala Ala Ala  Arg Arg Ala
    1895                1900                1905

Leu Arg  Ser Leu Lys Ala Asn  Gln Pro Gln Val Pro  Asn Ser
    1910                1915                1920


<210> SEQ ID NO 5
<211> LENGTH: 1930
<212> TYPE: PRT
<213> ORGANISM: Pan troglodytes

<400> SEQUENCE: 5

Met Lys Asn Pro Ala Leu Gln Pro Leu Ser Met Ala Gly Leu Gln Leu
1               5                   10                  15

Met Thr Pro Ala Ser Ser Pro Met Gly Pro Phe Phe Gly Leu Pro Trp
            20                  25                  30

Gln Gln Glu Ala Ile His Asp Asn Ile Tyr Thr Pro Arg Lys Tyr Gln
        35                  40                  45

Val Glu Leu Leu Glu Ala Ala Leu Asp His Asn Thr Ile Val Cys Leu
    50                  55                  60

Asn Thr Gly Ser Gly Lys Thr Phe Ile Ala Val Leu Leu Thr Lys Glu
65                  70                  75                  80

Leu Ser Tyr Gln Ile Arg Gly Asp Phe Ser Arg Asn Gly Lys Arg Thr
                85                  90                  95

Val Phe Leu Val Asn Ser Ala Asn Gln Val Ala Gln Gln Val Ser Ala
            100                 105                 110

Val Arg Thr His Ser Asp Leu Lys Val Gly Glu Tyr Ser Asn Leu Glu
        115                 120                 125

Val Asn Ala Ser Trp Thr Lys Glu Arg Trp Asn Gln Glu Phe Thr Lys
    130                 135                 140
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His Gln Val Leu Ile Met Thr Cys Tyr Val Ala Leu Asn Val Leu Lys
145                 150                 155                 160

Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
                165                 170                 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
            180                 185                 190

Cys Glu Asn Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
        195                 200                 205

Ile Leu Asn Gly Lys Cys Asp Pro Glu Glu Leu Glu Glu Lys Ile Gln
    210                 215                 220

Lys Leu Glu Lys Ile Leu Lys Ser Asn Ala Glu Thr Ala Thr Asp Leu
225                 230                 235                 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
                245                 250                 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Glu Arg Leu Leu Met
            260                 265                 270

Glu Leu Glu Glu Ala Leu Asn Phe Ile Asn Asp Cys Asn Ile Ser Val
        275                 280                 285

His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
    290                 295                 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305                 310                 315                 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
                325                 330                 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Phe Leu
            340                 345                 350

Arg Lys Ile His Ala Leu Cys Glu Glu His Phe Ser Pro Ala Ser Leu
        355                 360                 365

Asp Leu Lys Phe Val Thr Pro Lys Val Ile Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Glu Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Asn Lys Gln Met Glu Ala
                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Ile Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Ile Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
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                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Val Asp Thr Gly Glu Ile Asp Ile Asp Pro Val Met Asp Asp Asp
        595                 600                 605

Asp Val Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp Gly Gly Pro Arg
    610                 615                 620

Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg Tyr Cys Ala Arg
625                 630                 635                 640

Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys Cys Arg Thr Arg
                645                 650                 655

Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr Leu Pro Ile Asn
            660                 665                 670

Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met Ser Cys Val Arg
        675                 680                 685

Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu Lys Leu His Lys
    690                 695                 700

Ile Gly Glu Leu Asp Asp His Leu Met Pro Val Gly Lys Glu Thr Val
705                 710                 715                 720

Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu Glu Thr Ser Val
                725                 730                 735

Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys Tyr Pro Lys Ala
            740                 745                 750

Ile Pro Glu Cys Leu Arg Asp Ser Tyr Pro Arg Pro Asp Gln Pro Cys
        755                 760                 765

Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro Leu Pro Asp Glu
    770                 775                 780

Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu Asp Thr Thr Arg
785                 790                 795                 800

Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln Ile Pro His Phe
                805                 810                 815

Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser Ile Glu Leu Lys
            820                 825                 830

Lys Ser Gly Phe Met Leu Ser Leu Gln Met Leu Glu Leu Ile Thr Arg
        835                 840                 845

Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu Glu Lys Pro Ala
    850                 855                 860

Leu Glu Phe Lys Pro Thr Asp Ala Asp Ser Ala Tyr Cys Val Leu Pro
865                 870                 875                 880

Leu Asn Val Val Asn Asp Ser Ser Thr Leu Asp Ile Asp Phe Lys Phe
                885                 890                 895

Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly Ile Pro Ser Thr
            900                 905                 910

Lys Tyr Thr Lys Glu Thr Pro Phe Val Phe Lys Leu Glu Asp Tyr Gln
        915                 920                 925

Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp Gln Pro His Arg
    930                 935                 940

Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro Leu Ser Lys Phe
945                 950                 955                 960

Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr Lys Thr Lys Tyr
                965                 970                 975

Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu Asp Val Asp His
            980                 985                 990
```

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Thr Ser Ser Arg Leu Asn Leu Leu  Thr Pro Arg His Leu  Asn Gln Lys
        995                 1000                 1005

Gly Lys  Ala Leu Pro Leu Ser  Ser Ala Glu Lys Arg  Lys Ala Lys
    1010                 1015                 1020

Trp Glu  Ser Leu Gln Asn Lys  Gln Ile Leu Val Pro  Glu Leu Cys
    1025                 1030                 1035

Ala Ile  His Pro Ile Pro Ala  Ser Leu Trp Arg Lys  Ala Val Cys
    1040                 1045                 1050

Leu Pro  Ser Ile Leu Tyr Arg  Leu His Cys Leu Leu  Thr Ala Glu
    1055                 1060                 1065

Glu Leu  Arg Ala Gln Thr Ala  Ser Asp Ala Gly Val  Gly Val Arg
    1070                 1075                 1080

Ser Leu  Pro Ala Asp Phe Arg  Tyr Pro Asn Leu Asp  Phe Gly Trp
    1085                 1090                 1095

Lys Lys  Ser Ile Asp Ser Lys  Ser Phe Ile Ser Ile  Ser Asn Ser
    1100                 1105                 1110

Ser Ser  Ala Glu Asn Asp Asn  Tyr Cys Lys His Ser  Thr Ile Val
    1115                 1120                 1125

Pro Glu  Asn Ala Ala His Gln  Gly Ala Asn Arg Thr  Ser Ser Leu
    1130                 1135                 1140

Glu Asn  His Asp Gln Met Ser  Val Asn Cys Arg Thr  Leu Leu Ser
    1145                 1150                 1155

Glu Ser  Pro Gly Lys Leu His  Val Glu Val Ser Ala  Asp Leu Thr
    1160                 1165                 1170

Ala Ile  Asn Gly Leu Ser Tyr  Asn Gln Asn Leu Ala  Asn Gly Ser
    1175                 1180                 1185

Tyr Asp  Leu Ala Asn Arg Asp  Phe Cys Gln Gly Asn  Gln Leu Asn
    1190                 1195                 1200

Tyr Tyr  Lys Gln Glu Ile Pro  Val Gln Pro Thr Thr  Ser Tyr Ser
    1205                 1210                 1215

Ile Gln  Asn Leu Tyr Ser Tyr  Glu Asn Gln Pro Gln  Pro Ser Asp
    1220                 1225                 1230

Glu Cys  Thr Leu Leu Ser Asn  Lys Tyr Leu Asp Gly  Asn Ala Asn
    1235                 1240                 1245

Lys Ser  Thr Ser Asp Gly Ser  Pro Val Met Ala Val  Met Pro Gly
    1250                 1255                 1260

Thr Thr  Asp Thr Ile Gln Val  Leu Lys Gly Arg Met  Asp Ser Glu
    1265                 1270                 1275

Gln Ser  Pro Ser Ile Gly Tyr  Ser Ser Arg Thr Leu  Gly Pro Asn
    1280                 1285                 1290

Pro Gly  Leu Ile Leu Gln Ala  Leu Thr Leu Ser Asn  Ala Ser Asp
    1295                 1300                 1305

Gly Phe  Asn Leu Glu Arg Leu  Glu Met Leu Gly Asp  Ser Phe Leu
    1310                 1315                 1320

Lys His  Ala Ile Thr Thr Tyr  Leu Phe Cys Thr Tyr  Pro Asp Ala
    1325                 1330                 1335

His Glu  Gly Arg Leu Ser Tyr  Met Arg Ser Lys Lys  Val Ser Asn
    1340                 1345                 1350

Cys Asn  Leu Tyr Arg Leu Gly  Lys Lys Lys Gly Leu  Pro Ser Arg
    1355                 1360                 1365

Met Val  Val Ser Ile Phe Asp  Pro Pro Val Asn Trp  Leu Pro Pro
    1370                 1375                 1380
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Gly Tyr  Val Val Asn Gln Asp  Lys Ser Asn Thr Asp  Lys Trp Glu
    1385                 1390                 1395

Lys Asp  Glu Met Thr Lys Asp  Cys Met Leu Ala Asn  Gly Lys Leu
    1400                 1405                 1410

Asp Glu  Asp Tyr Glu Glu Glu  Asp Glu Glu Glu Glu  Ser Leu Met
    1415                 1420                 1425

Trp Arg  Ala Pro Lys Glu Glu  Ala Asp Tyr Glu Asp  Asp Phe Leu
    1430                 1435                 1440

Glu Tyr  Asp Gln Glu His Ile  Arg Phe Ile Asp Asn  Met Leu Met
    1445                 1450                 1455

Gly Ser  Gly Ala Phe Val Lys  Lys Ile Ser Leu Ser  Pro Phe Ser
    1460                 1465                 1470

Thr Thr  Asp Ser Ala Tyr Glu  Trp Lys Met Pro Lys  Lys Ser Ser
    1475                 1480                 1485

Leu Gly  Ser Met Pro Phe Ser  Ser Asp Phe Glu Asp  Phe Asp Tyr
    1490                 1495                 1500

Ser Ser  Trp Asp Ala Met Cys  Tyr Leu Asp Pro Ser  Lys Ala Val
    1505                 1510                 1515

Glu Glu  Asp Asp Phe Val Val  Gly Phe Trp Asn Pro  Ser Glu Glu
    1520                 1525                 1530

Asn Cys  Gly Val Asp Thr Gly  Lys Gln Ser Ile Ser  Tyr Asp Leu
    1535                 1540                 1545

His Thr  Glu Gln Cys Ile Ala  Asp Lys Ser Ile Ala  Asp Cys Val
    1550                 1555                 1560

Glu Ala  Leu Leu Gly Cys Tyr  Leu Thr Ser Cys Gly  Glu Arg Ala
    1565                 1570                 1575

Ala Gln  Leu Phe Leu Cys Ser  Leu Gly Leu Lys Val  Leu Pro Val
    1580                 1585                 1590

Ile Lys  Arg Thr Asp Arg Glu  Lys Ala Leu Cys Pro  Thr Arg Glu
    1595                 1600                 1605

Asn Phe  Asn Ser Gln Gln Lys  Asn Leu Ser Val Ser  Cys Ala Ala
    1610                 1615                 1620

Ala Ser  Val Ala Ser Ser Arg  Ser Ser Val Leu Lys  Asp Ser Glu
    1625                 1630                 1635

Tyr Gly  Cys Leu Lys Ile Pro  Pro Arg Cys Met Phe  Asp His Pro
    1640                 1645                 1650

Asp Ala  Asp Lys Thr Leu Asn  His Leu Ile Ser Gly  Phe Glu Asn
    1655                 1660                 1665

Phe Glu  Lys Lys Ile Asn Tyr  Arg Phe Lys Asn Lys  Ala Tyr Leu
    1670                 1675                 1680

Leu Gln  Ala Phe Thr His Ala  Ser Tyr His Tyr Asn  Thr Ile Thr
    1685                 1690                 1695

Asp Cys  Tyr Gln Arg Leu Glu  Phe Leu Gly Asp Ala  Ile Leu Asp
    1700                 1705                 1710

Tyr Leu  Ile Thr Lys His Leu  Tyr Glu Asp Pro Arg  Gln His Ser
    1715                 1720                 1725

Pro Gly  Val Leu Thr Asp Leu  Arg Ser Ala Leu Val  Asn Asn Thr
    1730                 1735                 1740

Ile Phe  Ala Ser Leu Ala Val  Lys Tyr Asp Tyr His  Lys Tyr Phe
    1745                 1750                 1755

Lys Ala  Val Ser Pro Glu Leu  Phe His Val Ile Asp  Asp Phe Val
    1760                 1765                 1770

Gln Phe  Gln Leu Glu Lys Asn  Glu Met Gln Gly Met  Asp Ser Glu
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    1775                1780                1785

Leu Arg  Arg Ser Glu Glu Asp  Glu Glu Lys Glu Glu  Asp Ile Glu
    1790                1795                1800

Val Pro  Lys Ala Met Gly Asp  Ile Phe Glu Ser Leu  Ala Gly Ala
    1805                1810                1815

Ile Tyr  Met Asp Ser Gly Met  Ser Leu Glu Thr Val  Trp Gln Val
    1820                1825                1830

Tyr Tyr  Pro Met Met Arg Pro  Leu Ile Glu Lys Phe  Ser Ala Asn
    1835                1840                1845

Val Pro  Arg Ser Pro Val Arg  Glu Leu Leu Glu Met  Glu Pro Glu
    1850                1855                1860

Thr Ala  Lys Phe Ser Pro Ala  Glu Arg Thr Tyr Asp  Gly Lys Val
    1865                1870                1875

Arg Val  Thr Val Glu Val Val  Gly Lys Gly Lys Phe  Lys Gly Val
    1880                1885                1890

Gly Arg  Ser Tyr Arg Ile Ala  Lys Ser Ala Ala Ala  Arg Arg Ala
    1895                1900                1905

Leu Arg  Ser Leu Lys Ala Asn  Gln Pro Gln Leu Trp  Val Ser Leu
    1910                1915                1920

Ala Leu  Pro Ser Thr Tyr Gln
    1925                1930


<210> SEQ ID NO 6
<211> LENGTH: 1923
<212> TYPE: PRT
<213> ORGANISM: Canis familiaris

<400> SEQUENCE: 6

Met Lys Ser Pro Ala Leu Gln Pro Leu Ser Met Ala Gly Leu Gln Leu
1               5                   10                  15

Met Thr Pro Ala Ser Ser Pro Met Gly Pro Phe Phe Gly Leu Pro Trp
            20                  25                  30

Gln Gln Glu Ala Ile His Asp Asn Ile Tyr Thr Pro Arg Lys Tyr Gln
        35                  40                  45

Val Glu Leu Leu Glu Ala Ala Leu Asp His Asn Thr Ile Val Cys Leu
    50                  55                  60

Asn Thr Gly Ser Gly Lys Thr Phe Ile Ala Val Leu Leu Thr Lys Glu
65                  70                  75                  80

Leu Ser Tyr Gln Ile Arg Gly Asp Phe Asn Arg Asn Gly Lys Arg Thr
                85                  90                  95

Val Phe Leu Val Asn Ser Ala Asn Gln Val Ala Gln Gln Val Ser Ala
            100                 105                 110

Val Arg Thr His Ser Asp Leu Lys Val Gly Glu Tyr Ser Asn Leu Glu
        115                 120                 125

Val Asn Ala Ser Trp Thr Lys Glu Lys Trp Asn Gln Glu Phe Thr Lys
    130                 135                 140

His Gln Val Leu Val Met Thr Cys Tyr Val Ala Leu Asn Val Leu Lys
145                 150                 155                 160

Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
                165                 170                 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
            180                 185                 190

Cys Glu Asn Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
        195                 200                 205
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Ile Leu Asn Gly Lys Cys Asp Pro Glu Glu Leu Glu Glu Lys Ile Gln
    210                 215                 220

Lys Leu Glu Lys Ile Leu Lys Ser Asn Ala Glu Thr Ala Thr Asp Leu
225                 230                 235                 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
                245                 250                 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Gly Arg Leu Leu Val
            260                 265                 270

Glu Leu Glu Glu Ala Leu Asn Phe Ile Asn Asp Cys Asn Ile Ser Val
        275                 280                 285

His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
    290                 295                 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305                 310                 315                 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
                325                 330                 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Phe Leu
            340                 345                 350

Arg Lys Ile His Ala Leu Cys Glu Glu His Phe Ser Pro Ala Ser Leu
        355                 360                 365

Asp Leu Lys Phe Val Thr Pro Lys Val Ile Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Glu Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Asn Lys Gln Met Glu Ala
                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Ile Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Ile Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Val Asp Thr Gly Glu Thr Asp Ile Glu Pro Val Val Asp Asp Asp
        595                 600                 605

Asp Val Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp Gly Gly Pro Arg
    610                 615                 620

Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg Tyr Cys Ala Arg
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625                 630                 635                 640

Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys Cys Arg Thr Arg
                645                 650                 655

Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr Leu Pro Ile Asn
            660                 665                 670

Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met Ser Cys Val Arg
        675                 680                 685

Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu Lys Leu His Lys
    690                 695                 700

Ile Gly Glu Leu Asp Asp His Leu Met Pro Val Gly Lys Glu Thr Val
705                 710                 715                 720

Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu Glu Thr Ser Val
                725                 730                 735

Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys Tyr Pro Lys Ala
            740                 745                 750

Ile Pro Glu Cys Leu Arg Asp Ser Tyr Pro Lys Pro Asp Gln Pro Cys
        755                 760                 765

Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro Leu Pro Asp Glu
    770                 775                 780

Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu Asp Thr Thr Arg
785                 790                 795                 800

Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln Ile Pro His Phe
                805                 810                 815

Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser Ile Glu Leu Lys
            820                 825                 830

Lys Ser Gly Phe Thr Leu Ser Leu Gln Met Leu Glu Leu Ile Thr Arg
        835                 840                 845

Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu Glu Lys Pro Ala
    850                 855                 860

Leu Glu Phe Lys Pro Thr Asp Ala Asp Ser Ala Tyr Cys Val Leu Pro
865                 870                 875                 880

Leu Asn Val Val Asn Asp Ser Ser Thr Leu Asp Ile Asp Phe Lys Phe
                885                 890                 895

Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly Ile Pro Ser Thr
            900                 905                 910

Lys Tyr Ser Lys Glu Thr Pro Phe Val Phe Lys Leu Glu Asp Tyr Gln
        915                 920                 925

Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp Gln Pro His Arg
    930                 935                 940

Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro Leu Ser Lys Phe
945                 950                 955                 960

Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr Lys Thr Lys Tyr
                965                 970                 975

Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu Asp Val Asp His
            980                 985                 990

Thr Ser Ser Arg Leu Asn Leu Leu  Thr Pro Arg His Leu  Asn Gln Lys
        995                 1000                1005

Gly Lys  Ala Leu Pro Leu Ser  Ser Ala Glu Lys Arg  Lys Ala Lys
    1010                1015                1020

Trp Glu  Ser Leu Gln Asn Lys  Gln Ile Leu Val Pro  Glu Leu Cys
    1025                1030                1035

Ala Ile  His Pro Ile Pro Ala  Ser Leu Trp Arg Lys  Ala Val Cys
    1040                1045                1050
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Leu Pro  Ser Ile Leu Tyr Arg  Leu His Cys Leu Leu  Thr Ala Glu
    1055                 1060                 1065

Glu Leu  Arg Ala Gln Thr Ala  Ser Asp Ala Gly Val  Gly Val Arg
    1070                 1075                 1080

Ser Leu  Pro Val Asp Phe Arg  Tyr Pro Asn Leu Asp  Phe Gly Trp
    1085                 1090                 1095

Lys Lys  Ser Ile Asp Ser Lys  Ser Phe Ile Ser Val  Ala Asn Ser
    1100                 1105                 1110

Ser Ser  Ala Glu Asn Glu Asn  Tyr Cys Lys His Ser  Thr Ile Val
    1115                 1120                 1125

Val Pro  Glu Asn Ala Ala Arg  Gln Gly Ala Asn Arg  Thr Ser Ser
    1130                 1135                 1140

Leu Glu  Asn His Asp Gln Met  Ser Val Asn Cys Arg  Thr Leu Phe
    1145                 1150                 1155

Ser Glu  Ser Pro Gly Lys Leu  Gln Ile Glu Val Val  Thr Asp Leu
    1160                 1165                 1170

Thr Ala  Ile Asn Gly Leu Ser  Tyr Asn Lys Asn Leu  Ala Asn Gly
    1175                 1180                 1185

Ser Tyr  Asp Leu Ala Asn Arg  Asp Phe Cys Gln Gly  Asn Gln Leu
    1190                 1195                 1200

Asn Tyr  Tyr Lys Gln Glu Ile  Pro Val Gln Pro Thr  Thr Ser Tyr
    1205                 1210                 1215

Pro Ile  Gln Asn Leu Tyr Asn  Tyr Glu Asn Gln Pro  Lys Pro Ser
    1220                 1225                 1230

Asp Glu  Cys Thr Leu Leu Ser  Asn Lys Tyr Leu Asp  Gly Asn Ala
    1235                 1240                 1245

Asn Lys  Ser Thr Ser Asp Gly  Ser Pro Thr Thr Ala  Ala Met Pro
    1250                 1255                 1260

Gly Thr  Thr Glu Ala Val Arg  Ala Leu Lys Asp Lys  Met Gly Ser
    1265                 1270                 1275

Glu Gln  Ser Pro Cys Pro Gly  Tyr Ser Ser Arg Thr  Leu Gly Pro
    1280                 1285                 1290

Asn Pro  Gly Leu Ile Leu Gln  Ala Leu Thr Leu Ser  Asn Ala Ser
    1295                 1300                 1305

Asp Gly  Phe Asn Leu Glu Arg  Leu Glu Met Leu Gly  Asp Ser Phe
    1310                 1315                 1320

Leu Lys  His Ala Ile Thr Thr  Tyr Leu Phe Cys Thr  Tyr Pro Asp
    1325                 1330                 1335

Ala His  Glu Gly Arg Leu Ser  Tyr Met Arg Ser Lys  Lys Val Ser
    1340                 1345                 1350

Asn Cys  Asn Leu Tyr Arg Leu  Gly Lys Lys Lys Gly  Leu Pro Ser
    1355                 1360                 1365

Arg Met  Val Val Ser Ile Phe  Asp Pro Pro Val Asn  Trp Leu Pro
    1370                 1375                 1380

Pro Gly  Tyr Val Val Asn Gln  Asp Lys Ser Asn Ala  Asp Lys Trp
    1385                 1390                 1395

Glu Lys  Asp Glu Met Thr Lys  Asp Cys Met Leu Ala  Asn Gly Lys
    1400                 1405                 1410

Leu Asp  Glu Asp Phe Glu Glu  Asp Asp Glu Glu Glu  Glu Asp Leu
    1415                 1420                 1425

Met Trp  Arg Ala Pro Lys Glu  Asp Ala Asp Tyr Glu  Asp Asp Phe
    1430                 1435                 1440
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Leu Glu Tyr Asp Gln Glu His Ile Lys Phe Ile Asp Asn Met Leu
1445 1450 1455

Met Gly Ser Gly Ala Phe Val Lys Lys Ile Ser Leu Ser Pro Phe
1460 1465 1470

Ser Thr Thr Asp Ser Ala Tyr Glu Trp Lys Met Pro Lys Lys Ser
1475 1480 1485

Ser Leu Gly Ser Met Pro Phe Ser Ser Asp Phe Glu Asp Phe Asp
1490 1495 1500

Tyr Ser Ser Trp Asp Ala Met Cys Tyr Leu Asp Pro Ser Lys Ala
1505 1510 1515

Val Glu Glu Asp Asp Phe Val Val Gly Phe Trp Asn Pro Ser Glu
1520 1525 1530

Glu Asn Cys Gly Val Asp Thr Gly Lys Gln Ser Ile Ser Tyr Asp
1535 1540 1545

Leu His Thr Glu Gln Cys Ile Ala Asp Lys Ser Ile Ala Asp Cys
1550 1555 1560

Val Glu Ala Leu Leu Gly Cys Tyr Leu Thr Ser Cys Gly Glu Arg
1565 1570 1575

Ala Ala Gln Leu Phe Leu Cys Ser Leu Gly Leu Lys Val Leu Pro
1580 1585 1590

Val Met Lys Arg Thr Asp Arg Glu Lys Thr Met Cys Pro Pro Arg
1595 1600 1605

Glu Asn Phe Ser Ser Gln Gln Lys Asn Leu Ser Gly Gly Arg Ala
1610 1615 1620

Ala Ala Ser Val Ala Ser Leu Arg Pro Ser Val Leu Lys Asp Leu
1625 1630 1635

Glu Tyr Gly Cys Leu Lys Ile Pro Pro Arg Cys Met Phe Asp His
1640 1645 1650

Pro Asp Ala Asp Lys Thr Leu Asn His Leu Ile Ser Gly Phe Glu
1655 1660 1665

Asn Phe Glu Lys Lys Ile Asn Tyr Arg Phe Lys Asn Lys Ala Tyr
1670 1675 1680

Leu Leu Gln Ala Phe Thr His Ala Ser Tyr His Tyr Asn Thr Ile
1685 1690 1695

Thr Asp Cys Tyr Gln Arg Leu Glu Phe Leu Gly Asp Ala Ile Leu
1700 1705 1710

Asp Tyr Leu Ile Thr Lys His Leu Tyr Glu Asp Pro Arg Gln His
1715 1720 1725

Ser Pro Gly Val Leu Thr Asp Leu Arg Ser Ala Leu Val Asn Asn
1730 1735 1740

Thr Ile Phe Ala Ser Leu Ala Val Lys Tyr Asp Tyr His Lys Tyr
1745 1750 1755

Phe Lys Ala Val Ser Pro Glu Leu Phe His Val Ile Asp Asp Phe
1760 1765 1770

Val Gln Phe Gln Leu Glu Lys Asn Glu Met Gln Gly Met Asp Ser
1775 1780 1785

Glu Leu Arg Arg Ser Glu Glu Asp Glu Glu Lys Glu Glu Asp Ile
1790 1795 1800

Glu Val Pro Lys Ala Met Gly Asp Ile Phe Glu Ser Leu Ala Gly
1805 1810 1815

Ala Ile Tyr Met Asp Ser Gly Met Ser Leu Glu Met Val Trp Gln
1820 1825 1830

Val Tyr Tyr Pro Met Met Arg Pro Leu Ile Glu Lys Phe Ser Ala

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    1835                1840                1845

Asn Val  Pro Arg Ser Pro Val  Arg Glu Leu Leu Glu  Met Glu Pro
    1850                1855                1860

Glu Thr  Ala Lys Phe Ser Pro  Ala Glu Arg Thr Tyr  Asp Gly Lys
    1865                1870                1875

Val Arg  Val Thr Val Glu Val  Val Gly Lys Gly Lys  Phe Lys Gly
    1880                1885                1890

Val Gly  Arg Ser Tyr Arg Ile  Ala Lys Ser Ala Ala  Ala Arg Arg
    1895                1900                1905

Ala Leu  Arg Ser Leu Lys Ala  Asn Gln Pro Gln Val  Pro Asn Ser
    1910                1915                1920


<210> SEQ ID NO 7
<211> LENGTH: 1918
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 7

Met Lys Ser Pro Ala Leu Gln Pro Leu Ser Met Ala Gly Leu Gln Leu
1               5                   10                  15

Met Thr Pro Ala Ser Ser Pro Met Gly Pro Phe Phe Gly Leu Pro Trp
            20                  25                  30

Gln Gln Glu Ala Ile His Asp Asn Ile Tyr Thr Pro Arg Lys Tyr Gln
        35                  40                  45

Val Glu Leu Leu Glu Ala Ala Leu Asp His Asn Thr Ile Val Cys Leu
    50                  55                  60

Asn Thr Gly Ser Gly Lys Thr Phe Ile Ala Val Leu Leu Thr Lys Glu
65                  70                  75                  80

Leu Ala His Gln Ile Arg Gly Asp Leu Ser Pro His Ala Lys Arg Thr
                85                  90                  95

Val Phe Leu Val Asn Ser Ala Asn Gln Val Ala Gln Gln Val Ser Ala
            100                 105                 110

Val Arg Thr His Ser Asp Leu Lys Val Gly Glu Tyr Ser Asn Leu Glu
        115                 120                 125

Val Asn Ala Ser Trp Thr Lys Glu Arg Trp Ser Gln Glu Phe Thr Lys
    130                 135                 140

His Gln Val Leu Ile Met Thr Cys Tyr Val Ala Leu Asn Val Leu Lys
145                 150                 155                 160

Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
                165                 170                 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
            180                 185                 190

Cys Asp Ser Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
        195                 200                 205

Ile Leu Asn Gly Lys Cys Asp Pro Asp Glu Leu Glu Glu Lys Ile Gln
    210                 215                 220

Lys Leu Glu Lys Ile Leu Lys Ser Gly Ala Glu Thr Ala Thr Asp Leu
225                 230                 235                 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
                245                 250                 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Glu Arg Leu Leu Met
            260                 265                 270

Glu Leu Glu Glu Ala Leu Asp Phe Ile Asn Asp Cys Asn Val Ser Val
        275                 280                 285
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His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
    290                 295                 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305                 310                 315                 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
                325                 330                 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Leu Leu
            340                 345                 350

Arg Lys Ile His Ala Leu Cys Glu Glu Tyr Phe Ser Pro Ala Ser Leu
        355                 360                 365

Asp Leu Lys Tyr Val Thr Pro Lys Val Met Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Asp Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Ser Lys Gln Met Glu Ala
                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Val Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Val Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Val Asp Gly Ala Glu Ala Asp Val His Ala Val Val Asp Asp Asp
        595                 600                 605

Asp Val Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp Gly Gly Pro Arg
    610                 615                 620

Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg Tyr Cys Ala Arg
625                 630                 635                 640

Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys Cys Arg Thr Arg
                645                 650                 655

Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr Leu Pro Ile Asn
            660                 665                 670

Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met Gly Cys Val Arg
        675                 680                 685

Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu Lys Leu His Lys
    690                 695                 700

Ile Gly Glu Leu Asp Glu His Leu Met Pro Val Gly Lys Glu Thr Val
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705                 710                 715                 720

Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu Glu Thr Ser Val
                725                 730                 735

Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys Tyr Pro Lys Ala
            740                 745                 750

Ile Pro Glu Cys Leu Arg Glu Ser Tyr Pro Lys Pro Asp Gln Pro Cys
        755                 760                 765

Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro Leu Pro Asp Glu
    770                 775                 780

Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu Asp Thr Thr Arg
785                 790                 795                 800

Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln Ile Pro His Phe
                805                 810                 815

Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser Ile Glu Leu Lys
            820                 825                 830

Lys Ser Gly Phe Thr Leu Ser Gln Gln Met Leu Glu Leu Val Thr Arg
        835                 840                 845

Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu Glu Lys Pro Ala
    850                 855                 860

Leu Glu Phe Gln Pro Ala Gly Ala Glu Ser Ala Tyr Cys Val Leu Pro
865                 870                 875                 880

Leu Asn Val Val Asn Asp Ser Ser Thr Leu Asp Ile Asp Phe Lys Phe
                885                 890                 895

Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly Ile Pro Ser Thr
            900                 905                 910

Lys Tyr Ser Lys Glu Thr Pro Phe Val Phe Lys Leu Glu Asp Tyr Gln
        915                 920                 925

Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp Gln Pro His Arg
    930                 935                 940

Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro Leu Ser Lys Phe
945                 950                 955                 960

Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr Lys Thr Lys Tyr
                965                 970                 975

Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu Asp Val Asp His
            980                 985                 990

Thr Ser Ser Arg Leu Asn Leu Leu  Thr Pro Arg His Leu  Asn Gln Lys
        995                 1000                 1005

Gly Lys  Ala Leu Pro Leu Ser  Ser Ala Glu Lys Arg  Lys Ala Lys
    1010                 1015                 1020

Trp Glu  Ser Leu Gln Asn Lys  Gln Ile Leu Val Pro  Glu Leu Cys
    1025                 1030                 1035

Ala Ile  His Pro Ile Pro Ala  Ser Leu Trp Arg Lys  Ala Val Cys
    1040                 1045                 1050

Leu Pro  Ser Ile Leu Tyr Arg  Leu His Cys Leu Leu  Thr Ala Glu
    1055                 1060                 1065

Glu Leu  Arg Ala Gln Thr Ala  Ser Asp Ala Gly Val  Gly Val Arg
    1070                 1075                 1080

Ser Leu  Pro Ala Asp Phe Arg  Tyr Pro Asn Leu Asp  Phe Gly Trp
    1085                 1090                 1095

Lys Lys  Ser Ile Asp Ser Lys  Ser Phe Ile Ser Thr  Cys Asn Ser
    1100                 1105                 1110

Ser Leu  Ala Glu Ser Asp Asn  Tyr Cys Lys His Ser  Thr Thr Val
    1115                 1120                 1125
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Val Pro  Glu Asn Ala Ala His  Gln Gly Ala Thr Arg  Pro Ser Leu
    1130                 1135                 1140

Glu Asn  His Asp Gln Met Ser  Val Asn Cys Lys Arg  Leu Pro Ala
    1145                 1150                 1155

Glu Ser  Pro Ala Lys Leu Gln  Ser Glu Val Ser Val  Asp Leu Thr
    1160                 1165                 1170

Ala Ile  Asn Gly Leu Ser Tyr  Asn Lys Ser Leu Ala  Asn Gly Ser
    1175                 1180                 1185

Tyr Asp  Leu Val Asn Arg Asp  Phe Cys Gln Gly Asn  Gln Leu Thr
    1190                 1195                 1200

Tyr Phe  Lys Gln Glu Ile Pro  Val Gln Pro Thr Thr  Ser Tyr Pro
    1205                 1210                 1215

Ile Gln  Asn Leu Tyr Asn Tyr  Glu Asn Gln Pro Thr  Pro Ser Asn
    1220                 1225                 1230

Glu Cys  Pro Leu Leu Ser Asn  Lys Tyr Leu Asp Gly  Asn Ala Asn
    1235                 1240                 1245

Thr Ser  Thr Ser Asp Gly Ser  Pro Ala Gly Ser Pro  Arg Pro Ala
    1250                 1255                 1260

Met Met  Thr Ala Val Glu Ala  Leu Glu Gly Arg Thr  Asp Ser Glu
    1265                 1270                 1275

Gln Ser  Pro Ser Val Gly His  Ser Ser Arg Thr Leu  Gly Pro Asn
    1280                 1285                 1290

Pro Gly  Leu Ile Leu Gln Ala  Leu Thr Leu Ser Asn  Ala Ser Asp
    1295                 1300                 1305

Gly Phe  Asn Leu Glu Arg Leu  Glu Met Leu Gly Asp  Ser Phe Leu
    1310                 1315                 1320

Lys His  Ala Ile Thr Thr Tyr  Leu Phe Cys Thr Tyr  Pro Asp Ala
    1325                 1330                 1335

His Glu  Gly Arg Leu Ser Tyr  Met Arg Ser Lys Lys  Val Ser Asn
    1340                 1345                 1350

Cys Asn  Leu Tyr Arg Leu Gly  Lys Lys Gln Gly Leu  Pro Ser Arg
    1355                 1360                 1365

Met Val  Val Ser Ile Phe Asp  Pro Pro Val Asn Trp  Leu Pro Pro
    1370                 1375                 1380

Gly Tyr  Val Val Asn Gln Asp  Lys Ser Asn Ser Glu  Lys Trp Glu
    1385                 1390                 1395

Lys Asp  Glu Met Thr Lys Asp  Cys Leu Leu Ala Asn  Gly Lys Leu
    1400                 1405                 1410

Gly Glu  Asp Cys Glu Glu Glu  Glu Glu Glu Glu Leu  Ala Trp Arg
    1415                 1420                 1425

Ala Pro  Lys Glu Glu Ala Glu  Tyr Glu Asp Asp Leu  Leu Glu Tyr
    1430                 1435                 1440

Asp Gln  Glu His Ile Gln Phe  Ile Asp Ser Met Leu  Met Gly Ser
    1445                 1450                 1455

Gly Ala  Phe Val Lys Lys Ile  Pro Leu Ser Pro Phe  Ser Thr Ser
    1460                 1465                 1470

Asp Ser  Ala Tyr Glu Trp Lys  Met Pro Lys Lys Ala  Ser Leu Gly
    1475                 1480                 1485

Ser Val  Pro Phe Ser Ser Asp  Leu Glu Asp Phe Asp  Tyr Ser Ser
    1490                 1495                 1500

Trp Asp  Ala Met Cys Tyr Leu  Asp Pro Ser Lys Ala  Val Glu Glu
    1505                 1510                 1515
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Asp Asp  Phe Val Val Gly Phe  Trp Asn Pro Ser Glu  Glu Asn Cys
    1520                 1525                 1530

Gly Val  Asp Thr Gly Lys Gln  Ser Ile Ser Tyr Asp  Leu His Thr
    1535                 1540                 1545

Glu Gln  Cys Ile Ala Asp Lys  Ser Ile Ala Asp Cys  Val Glu Ala
    1550                 1555                 1560

Leu Leu  Gly Cys Tyr Leu Thr  Ser Cys Gly Glu Arg  Ala Ala Gln
    1565                 1570                 1575

Leu Phe  Leu Cys Ser Leu Gly  Leu Lys Val Leu Pro  Val Ile Lys
    1580                 1585                 1590

Arg Thr  Ser Arg Asp Lys Ala  Ser Tyr Pro Ala Gln  Glu Asn Ser
    1595                 1600                 1605

Ser Ser  Gln Gln Lys Ser Pro  Ser Gly Ser Cys Ala  Ala Ala Val
    1610                 1615                 1620

Ser Pro  Arg Ser Ser Ala Gly  Lys Asp Leu Glu Tyr  Gly Cys Leu
    1625                 1630                 1635

Lys Ile  Pro Pro Arg Cys Met  Phe Asp His Pro Asp  Ala Glu Lys
    1640                 1645                 1650

Thr Leu  Asn His Leu Ile Ser  Gly Phe Glu Asn Phe  Glu Lys Lys
    1655                 1660                 1665

Ile Asn  Tyr Ile Phe Lys Asn  Lys Ala Tyr Leu Leu  Gln Ala Phe
    1670                 1675                 1680

Thr His  Ala Ser Tyr His Tyr  Asn Thr Ile Thr Asp  Cys Tyr Gln
    1685                 1690                 1695

Arg Leu  Glu Phe Leu Gly Asp  Ala Ile Leu Asp Tyr  Leu Ile Thr
    1700                 1705                 1710

Lys His  Leu Tyr Glu Asp Pro  Arg Gln His Ser Pro  Gly Val Leu
    1715                 1720                 1725

Thr Asp  Leu Arg Ser Ala Leu  Val Asn Asn Thr Ile  Phe Ala Ser
    1730                 1735                 1740

Leu Ala  Val Lys Tyr Asp Tyr  His Lys Tyr Phe Lys  Ala Val Ser
    1745                 1750                 1755

Pro Glu  Leu Phe His Val Ile  Asp Asp Phe Val Gln  Phe Gln Leu
    1760                 1765                 1770

Glu Lys  Asn Glu Met Gln Gly  Met Asp Ser Glu Leu  Arg Arg Ser
    1775                 1780                 1785

Glu Glu  Asp Glu Glu Lys Glu  Glu Asp Ile Glu Val  Pro Lys Ala
    1790                 1795                 1800

Met Gly  Asp Ile Phe Glu Ser  Leu Ala Gly Ala Ile  Tyr Met Asp
    1805                 1810                 1815

Ser Gly  Met Ser Leu Glu Val  Val Trp Gln Val Tyr  Tyr Pro Met
    1820                 1825                 1830

Met Arg  Pro Leu Ile Glu Lys  Phe Ser Ala Asn Val  Pro Arg Ser
    1835                 1840                 1845

Pro Val  Arg Glu Leu Leu Glu  Met Glu Pro Glu Thr  Ala Lys Phe
    1850                 1855                 1860

Ser Pro  Ala Glu Arg Thr Tyr  Asp Gly Lys Val Arg  Val Thr Val
    1865                 1870                 1875

Glu Val  Val Gly Lys Gly Lys  Phe Lys Gly Val Gly  Arg Ser Tyr
    1880                 1885                 1890

Arg Ile  Ala Lys Ser Ala Ala  Ala Arg Arg Ala Leu  Arg Ser Leu
    1895                 1900                 1905

Lys Ala  Asn Gln Pro Leu Val  Pro Asn Ser
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1910 1915

<210> SEQ ID NO 8
<211> LENGTH: 1916
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 8

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1 5 10 15

Met Thr Pro Ala Ser Ser Pro Met Gly Pro Phe Phe Gly Leu Pro Trp
20 25 30

Gln Gln Glu Ala Ile His Asp Asn Ile Tyr Thr Pro Arg Lys Tyr Gln
35 40 45

Val Glu Leu Leu Glu Ala Ala Leu Asp His Asn Thr Ile Val Cys Leu
50 55 60

Asn Thr Gly Ser Gly Lys Thr Phe Ile Ala Val Leu Leu Thr Lys Glu
65 70 75 80

Leu Ala His Gln Ile Arg Gly Asp Leu Asn Pro His Ala Lys Arg Thr
85 90 95

Val Phe Leu Val Asn Ser Ala Asn Gln Val Ala Gln Gln Val Ser Ala
100 105 110

Val Arg Thr His Ser Asp Leu Lys Val Gly Glu Tyr Ser Asp Leu Glu
115 120 125

Val Asn Ala Ser Trp Thr Lys Glu Arg Trp Ser Gln Glu Phe Thr Lys
130 135 140

His Gln Val Leu Ile Met Thr Cys Tyr Val Ala Leu Thr Val Leu Lys
145 150 155 160

Asn Gly Tyr Leu Ser Leu Ser Asp Ile Asn Leu Leu Val Phe Asp Glu
165 170 175

Cys His Leu Ala Ile Leu Asp His Pro Tyr Arg Glu Ile Met Lys Leu
180 185 190

Cys Glu Ser Cys Pro Ser Cys Pro Arg Ile Leu Gly Leu Thr Ala Ser
195 200 205

Ile Leu Asn Gly Lys Cys Asp Pro Glu Glu Leu Glu Glu Lys Ile Gln
210 215 220

Lys Leu Glu Arg Ile Leu Arg Ser Asp Ala Glu Thr Ala Thr Asp Leu
225 230 235 240

Val Val Leu Asp Arg Tyr Thr Ser Gln Pro Cys Glu Ile Val Val Asp
245 250 255

Cys Gly Pro Phe Thr Asp Arg Ser Gly Leu Tyr Glu Arg Leu Leu Met
260 265 270

Glu Leu Glu Ala Ala Leu Asp Phe Ile Asn Asp Cys Asn Val Ala Val
275 280 285

His Ser Lys Glu Arg Asp Ser Thr Leu Ile Ser Lys Gln Ile Leu Ser
290 295 300

Asp Cys Arg Ala Val Leu Val Val Leu Gly Pro Trp Cys Ala Asp Lys
305 310 315 320

Val Ala Gly Met Met Val Arg Glu Leu Gln Lys Tyr Ile Lys His Glu
325 330 335

Gln Glu Glu Leu His Arg Lys Phe Leu Leu Phe Thr Asp Thr Leu Leu
340 345 350

Arg Lys Ile His Ala Leu Cys Glu Glu Tyr Phe Ser Pro Ala Ser Leu
355 360 365

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Asp Leu Lys Tyr Val Thr Pro Lys Val Met Lys Leu Leu Glu Ile Leu
    370                 375                 380

Arg Lys Tyr Lys Pro Tyr Glu Arg Gln Gln Phe Glu Ser Val Glu Trp
385                 390                 395                 400

Tyr Asn Asn Arg Asn Gln Asp Asn Tyr Val Ser Trp Ser Asp Ser Glu
                405                 410                 415

Asp Asp Asp Asp Asp Glu Glu Ile Glu Glu Lys Glu Lys Pro Glu Thr
            420                 425                 430

Asn Phe Pro Ser Pro Phe Thr Asn Ile Leu Cys Gly Ile Ile Phe Val
        435                 440                 445

Glu Arg Arg Tyr Thr Ala Val Val Leu Asn Arg Leu Ile Lys Glu Ala
    450                 455                 460

Gly Lys Gln Asp Pro Glu Leu Ala Tyr Ile Ser Ser Asn Phe Ile Thr
465                 470                 475                 480

Gly His Gly Ile Gly Lys Asn Gln Pro Arg Ser Lys Gln Met Glu Ala
                485                 490                 495

Glu Phe Arg Lys Gln Glu Glu Val Leu Arg Lys Phe Arg Ala His Glu
            500                 505                 510

Thr Asn Leu Leu Ile Ala Thr Ser Val Val Glu Glu Gly Val Asp Ile
        515                 520                 525

Pro Lys Cys Asn Leu Val Val Arg Phe Asp Leu Pro Thr Glu Tyr Arg
    530                 535                 540

Ser Tyr Val Gln Ser Lys Gly Arg Ala Arg Ala Pro Ile Ser Asn Tyr
545                 550                 555                 560

Val Met Leu Ala Asp Thr Asp Lys Ile Lys Ser Phe Glu Glu Asp Leu
                565                 570                 575

Lys Thr Tyr Lys Ala Ile Glu Lys Ile Leu Arg Asn Lys Cys Ser Lys
            580                 585                 590

Ser Ala Asp Gly Ala Glu Ala Asp Val His Ala Gly Val Asp Asp Glu
        595                 600                 605

Asp Ala Phe Pro Pro Tyr Val Leu Arg Pro Asp Asp Gly Gly Pro Arg
    610                 615                 620

Val Thr Ile Asn Thr Ala Ile Gly His Ile Asn Arg Tyr Cys Ala Arg
625                 630                 635                 640

Leu Pro Ser Asp Pro Phe Thr His Leu Ala Pro Lys Cys Arg Thr Arg
                645                 650                 655

Glu Leu Pro Asp Gly Thr Phe Tyr Ser Thr Leu Tyr Leu Pro Ile Asn
            660                 665                 670

Ser Pro Leu Arg Ala Ser Ile Val Gly Pro Pro Met Asp Ser Val Arg
        675                 680                 685

Leu Ala Glu Arg Val Val Ala Leu Ile Cys Cys Glu Lys Leu His Lys
    690                 695                 700

Ile Gly Glu Leu Asp Glu His Leu Met Pro Val Gly Lys Glu Thr Val
705                 710                 715                 720

Lys Tyr Glu Glu Glu Leu Asp Leu His Asp Glu Glu Glu Thr Ser Val
                725                 730                 735

Pro Gly Arg Pro Gly Ser Thr Lys Arg Arg Gln Cys Tyr Pro Lys Ala
            740                 745                 750

Ile Pro Glu Cys Leu Arg Glu Ser Tyr Pro Lys Pro Asp Gln Pro Cys
        755                 760                 765

Tyr Leu Tyr Val Ile Gly Met Val Leu Thr Thr Pro Leu Pro Asp Glu
    770                 775                 780

Leu Asn Phe Arg Arg Arg Lys Leu Tyr Pro Pro Glu Asp Thr Thr Arg
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785                 790                 795                 800

Cys Phe Gly Ile Leu Thr Ala Lys Pro Ile Pro Gln Ile Pro His Phe
                805                 810                 815

Pro Val Tyr Thr Arg Ser Gly Glu Val Thr Ile Ser Ile Glu Leu Lys
            820                 825                 830

Lys Ser Gly Phe Thr Leu Ser Gln Gln Met Leu Glu Leu Ile Thr Arg
        835                 840                 845

Leu His Gln Tyr Ile Phe Ser His Ile Leu Arg Leu Glu Lys Pro Ala
    850                 855                 860

Leu Glu Phe Lys Pro Thr Gly Ala Glu Ser Ala Tyr Cys Val Leu Pro
865                 870                 875                 880

Leu Asn Val Val Asn Asp Ser Gly Thr Leu Asp Ile Asp Phe Lys Phe
                885                 890                 895

Met Glu Asp Ile Glu Lys Ser Glu Ala Arg Ile Gly Ile Pro Ser Thr
            900                 905                 910

Lys Tyr Ser Lys Glu Thr Pro Phe Val Phe Lys Leu Glu Asp Tyr Gln
        915                 920                 925

Asp Ala Val Ile Ile Pro Arg Tyr Arg Asn Phe Asp Gln Pro His Arg
    930                 935                 940

Phe Tyr Val Ala Asp Val Tyr Thr Asp Leu Thr Pro Leu Ser Lys Phe
945                 950                 955                 960

Pro Ser Pro Glu Tyr Glu Thr Phe Ala Glu Tyr Tyr Lys Thr Lys Tyr
                965                 970                 975

Asn Leu Asp Leu Thr Asn Leu Asn Gln Pro Leu Leu Asp Val Asp His
            980                 985                 990

Thr Ser Ser Arg Leu Asn Leu Leu  Thr Pro Arg His Leu  Asn Gln Lys
        995                 1000                1005

Gly Lys  Ala Leu Pro Leu Ser  Ser Ala Glu Lys Arg  Lys Ala Lys
    1010                1015                1020

Trp Glu  Ser Leu Gln Asn Lys  Gln Ile Leu Val Pro  Glu Leu Cys
    1025                1030                1035

Ala Ile  His Pro Ile Pro Ala  Ser Leu Trp Arg Lys  Ala Val Cys
    1040                1045                1050

Leu Pro  Ser Ile Leu Tyr Arg  Leu His Cys Leu Leu  Thr Ala Glu
    1055                1060                1065

Glu Leu  Arg Ala Gln Thr Ala  Ser Asp Ala Gly Val  Gly Val Arg
    1070                1075                1080

Ser Leu  Pro Val Asp Phe Arg  Tyr Pro Asn Leu Asp  Phe Gly Trp
    1085                1090                1095

Lys Lys  Ser Ile Asp Ser Lys  Ser Phe Ile Ser Ser  Cys Asn Ser
    1100                1105                1110

Ser Leu  Ala Glu Ser Asp Asn  Tyr Cys Lys His Ser  Thr Thr Val
    1115                1120                1125

Val Pro  Glu His Ala Ala His  Gln Gly Ala Thr Arg  Pro Ser Leu
    1130                1135                1140

Glu Asn  His Asp Gln Met Ser  Val Asn Cys Lys Arg  Leu Pro Ala
    1145                1150                1155

Glu Ser  Pro Ala Lys Leu Gln  Ser Glu Val Ser Thr  Asp Leu Thr
    1160                1165                1170

Ala Ile  Asn Gly Leu Ser Tyr  Asn Lys Asn Leu Ala  Asn Gly Ser
    1175                1180                1185

Tyr Asp  Leu Val Asn Arg Asp  Phe Cys Gln Gly Asn  Gln Leu Asn
    1190                1195                1200
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Tyr Phe  Lys Gln Glu Ile Pro  Val Gln Pro Thr Thr  Ser Tyr Pro
    1205                 1210                 1215

Ile Gln  Asn Leu Tyr Asn Tyr  Glu Asn Gln Pro Lys  Pro Ser Asn
    1220                 1225                 1230

Glu Cys  Pro Leu Leu Ser Asn  Thr Tyr Leu Asp Gly  Asn Ala Asn
    1235                 1240                 1245

Thr Ser  Thr Ser Asp Gly Ser  Pro Ala Val Ser Thr  Met Pro Ala
    1250                 1255                 1260

Met Met  Asn Ala Val Lys Ala  Leu Lys Asp Arg Met  Asp Ser Glu
    1265                 1270                 1275

Gln Ser  Pro Ser Val Gly Tyr  Ser Ser Arg Thr Leu  Gly Pro Asn
    1280                 1285                 1290

Pro Gly  Leu Ile Leu Gln Ala  Leu Thr Leu Ser Asn  Ala Ser Asp
    1295                 1300                 1305

Gly Phe  Asn Leu Glu Arg Leu  Glu Met Leu Gly Asp  Ser Phe Leu
    1310                 1315                 1320

Lys His  Ala Ile Thr Thr Tyr  Leu Phe Cys Thr Tyr  Pro Asp Ala
    1325                 1330                 1335

His Glu  Gly Arg Leu Ser Tyr  Met Arg Ser Lys Lys  Val Ser Asn
    1340                 1345                 1350

Cys Asn  Leu Tyr Arg Leu Gly  Lys Lys Lys Gly Leu  Pro Ser Arg
    1355                 1360                 1365

Met Val  Val Ser Ile Phe Asp  Pro Pro Val Asn Trp  Leu Pro Pro
    1370                 1375                 1380

Gly Tyr  Val Val Asn Gln Asp  Lys Ser Asn Ser Glu  Lys Trp Glu
    1385                 1390                 1395

Lys Asp  Glu Met Thr Lys Asp  Cys Leu Leu Ala Asn  Gly Lys Leu
    1400                 1405                 1410

Gly Glu  Ala Cys Glu Glu Glu  Glu Asp Leu Thr Trp  Arg Ala Pro
    1415                 1420                 1425

Lys Glu  Glu Ala Glu Asp Glu  Asp Asp Phe Leu Glu  Tyr Asp Gln
    1430                 1435                 1440

Glu His  Ile Gln Phe Ile Asp  Ser Met Leu Met Gly  Ser Gly Ala
    1445                 1450                 1455

Phe Val  Arg Lys Ile Ser Leu  Ser Pro Phe Ser Ala  Ser Asp Ser
    1460                 1465                 1470

Ala Tyr  Glu Trp Lys Met Pro  Lys Lys Ala Ser Leu  Gly Ser Met
    1475                 1480                 1485

Pro Phe  Ala Ser Gly Leu Glu  Asp Phe Asp Tyr Ser  Ser Trp Asp
    1490                 1495                 1500

Ala Met  Cys Tyr Leu Asp Pro  Ser Lys Ala Val Glu  Glu Asp Asp
    1505                 1510                 1515

Phe Val  Val Gly Phe Trp Asn  Pro Ser Glu Glu Asn  Cys Gly Val
    1520                 1525                 1530

Asp Thr  Gly Lys Gln Ser Ile  Ser Tyr Asp Leu His  Thr Glu Gln
    1535                 1540                 1545

Cys Ile  Ala Asp Lys Ser Ile  Ala Asp Cys Val Glu  Ala Leu Leu
    1550                 1555                 1560

Gly Cys  Tyr Leu Thr Ser Cys  Gly Glu Arg Ala Ala  Gln Leu Phe
    1565                 1570                 1575

Leu Cys  Ser Leu Gly Leu Lys  Val Leu Pro Val Ile  Lys Arg Thr
    1580                 1585                 1590
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Ser Arg  Glu Lys Ala Leu Asp  Pro Ala Gln Glu Asn  Gly Ser Ser
    1595                 1600                 1605

Gln Gln  Lys Ser Leu Ser Gly  Ser Cys Ala Ser Pro  Val Gly Pro
    1610                 1615                 1620

Arg Ser  Ser Ala Gly Lys Asp  Leu Glu Tyr Gly Cys  Leu Lys Ile
    1625                 1630                 1635

Pro Pro  Arg Cys Met Phe Asp  His Pro Asp Ala Glu  Lys Thr Leu
    1640                 1645                 1650

Asn His  Leu Ile Ser Gly Phe  Glu Thr Phe Glu Lys  Lys Ile Asn
    1655                 1660                 1665

Tyr Arg  Phe Lys Asn Lys Ala  Tyr Leu Leu Gln Ala  Phe Thr His
    1670                 1675                 1680

Ala Ser  Tyr His Tyr Asn Thr  Ile Thr Asp Cys Tyr  Gln Arg Leu
    1685                 1690                 1695

Glu Phe  Leu Gly Asp Ala Ile  Leu Asp Tyr Leu Ile  Thr Lys His
    1700                 1705                 1710

Leu Tyr  Glu Asp Pro Arg Gln  His Ser Pro Gly Val  Leu Thr Asp
    1715                 1720                 1725

Leu Arg  Ser Ala Leu Val Asn  Asn Thr Ile Phe Ala  Ser Leu Ala
    1730                 1735                 1740

Val Lys  Tyr Asp Tyr His Lys  Tyr Phe Lys Ala Val  Ser Pro Glu
    1745                 1750                 1755

Leu Phe  His Val Ile Asp Asp  Phe Val Lys Phe Gln  Leu Glu Lys
    1760                 1765                 1770

Asn Glu  Met Gln Gly Met Asp  Ser Glu Leu Arg Arg  Ser Glu Glu
    1775                 1780                 1785

Asp Glu  Glu Lys Glu Glu Asp  Ile Glu Val Pro Lys  Ala Met Gly
    1790                 1795                 1800

Asp Ile  Phe Glu Ser Leu Ala  Gly Ala Ile Tyr Met  Asp Ser Gly
    1805                 1810                 1815

Met Ser  Leu Glu Val Val Trp  Gln Val Tyr Tyr Pro  Met Met Gln
    1820                 1825                 1830

Pro Leu  Ile Glu Lys Phe Ser  Ala Asn Val Pro Arg  Ser Pro Val
    1835                 1840                 1845

Arg Glu  Leu Leu Glu Met Glu  Pro Glu Thr Ala Lys  Phe Ser Pro
    1850                 1855                 1860

Ala Glu  Arg Thr Tyr Asp Gly  Lys Val Arg Val Thr  Val Glu Val
    1865                 1870                 1875

Val Gly  Lys Gly Lys Phe Lys  Gly Val Gly Arg Ser  Tyr Arg Ile
    1880                 1885                 1890

Ala Lys  Ser Ala Ala Ala Arg  Arg Ala Leu Arg Ser  Leu Lys Ala
    1895                 1900                 1905

Asn Gln  Pro Gln Val Pro Asn  Ser
    1910                 1915
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<210> SEQ ID NO 9
<211> LENGTH: 73
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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 9

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ugagguagua gguuguauag uuuuaggguc acacccacca cugggagaua acuauacaau     60

cuacugucuu acc                                                        73
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uacaaccuac ugccucauu 79

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<213> ORGANISM: Artificial sequence
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<223> OTHER INFORMATION: Synthetic sequence

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uaaagugcuu auagugcagg uaguguguag ccaucuacug cauuacgagc acuuaaag 58

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<212> TYPE: RNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 17

uauacaaugu gcuagcuuuc u 21

<210> SEQ ID NO 18
<211> LENGTH: 21
<212> TYPE: RNA
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 18

aaagcuagca cauuguauag u 21

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<211> LENGTH: 9
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<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 19

Cys Leu Asn Thr Gly Ser Gly Lys Thr
1 5

<210> SEQ ID NO 20
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Artificial sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic sequence

<400> SEQUENCE: 20

Cys Leu Asn Asp Gly Ser Gly Lys Thr
1 5

<210> SEQ ID NO 21
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1               5


<210> SEQ ID NO 23
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Asp Glu Cys His
1
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What is claimed is:

1. A purified, enzymatically active Dicer complex comprising:
   a) a first polypeptide comprising an amino acid sequence having at least 85% sequence identity to:
      (i) amino acids 1-1008 of the amino acid sequence of SEQ ID NO: 1 comprising an ATPase/helicase domain, a DUF domain, and a PAZ domain,
      (ii) amino acids 1-1068 of the amino acid sequence of SEQ ID NO: 1 comprising an ATPase/helicase domain, a DUF domain, and a PAZ domain,
      (iii) amino acids 605-1008 of the amino acid sequence of SEQ ID NO: 1 comprising a DUF and a PAZ domain, or
      (iv) amino acids 605-1068 of the amino acid sequence of SEQ ID NO: 1 comprising a DUF and a PAZ domain,
      wherein said first polypeptide lacks an RNase IIIa domain and an RNase IIIb domain; and
   b) a second polypeptide comprising an amino acid sequence having at least 85% sequence identity to:
      (i) amino acids 1235 to 1922 of the amino acid sequence of SEQ ID NO: 1 comprising an RNaseIIIa domain, an RNaseIIIb domain, and a dsRBD domain,
      (ii) amino acids 1296 to 1922 of the amino acid sequence of SEQ ID NO:1 comprising an RNaseIIIa domain, an RNaseIIIb domain, and dsRBD domain,
      (iii) amino acids 1235 to 1772 of the amino acid sequence of SEQ ID NO:1 comprising an RNaseIIIa and an RNaseIIIb domain, or
      (iv) amino acids 1296 to 1772 of the amino acid sequence of SEQ ID NO:1 comprising an RNaseIIIa and an RNaseIIIb domain,
      wherein said second polypeptide lacks at least one of: a DUF domain and a PAZ domain,
   wherein said first polypeptide and said second polypeptide spontaneously associate to form an enzymatically active Dicer complex that has endoribonuclease activity.

2. The purified Dicer complex of claim **1**, wherein the first polypeptide comprises amino acids 1-604 of the amino acid sequence of SEQ ID NO: 1 (a DExD/H-box domain).

3. The purified Dicer complex of claim **1**, wherein the first polypeptide lacks amino acids 1-604 of the amino acid sequence of SEQ ID NO:1 (a DExD/H-box domain).

4. A composition comprising:
   a) the purified, enzymatically active Dicer complex of claim **1**; and
   b) a buffer.

5. A method of producing an siRNA, the method comprising contacting the purified Dicer complex of claim **1** with a double-stranded RNA (dsRNA) substrate, wherein the Dicer complex cleaves the dsRNA substrate, thereby producing an siRNA.

6. The method of claim **5**, wherein the siRNA has a length of from **21** to **23** nucleotides.

7. The purified Dicer complex of claim **1**, wherein the second polypeptide lacks a double-stranded RNA binding domain.

8. The purified Dicer complex of claim **1**, wherein the second polypeptide lacks a DUF domain and a PAZ domain.

9. The purified Dicer complex of claim **1**, wherein at least one of the first and second polypeptides comprises a heterologous polypeptide that provides for a detectable signal and/or facilitates protein purification or isolation.

* * * * *